

The Cost of Asking for the Moon

THE United States Environmental Protection Agency has only itself to blame for the situation in which Mr William D. Ruckelshaus, the administrator, has had to climb down about the 1975 regulations proposed for automobile exhaust emission (see page 491). Nobody will quarrel with the general aim of the regulations, promulgated under the terms of the Clean Air Act of 1972, that city air should be cleaner and that automobiles should dirty it less than they do at present. But the 1975 regulations were unrealistically stringent, the methods by which they were to be achieved were hardly the most suitable and even now there must remain serious doubt as to whether the mere postponement of the enforcement of the Clean Air Act on automobiles is wise, let alone practicable.

In these days when even students in elementary schools are being urged to take to heart the principle that a system is a system, it is absurd that the United States Administration and Congress between them should have embarked on the laudable cause of reducing the amount of pollution in the streets of city centres by imposing particular restrictions on the percentages of various pollutants to be permitted in the exhaust of all motor vehicles, wherever they happen to travel. The trouble is, of course, that there can be no assurance that the restrictions originally intended for 1975 will bring about the improvement of air quality desired. In the last resort, what matters is the question of how damaging are the concentrations of carbon monoxide, sulphur dioxide and nitrogen oxide expected to be on the health and welfare of people, and this is a topic on which more research is needed. To tell from what Mr Ruckelshaus said this week, there is a long way to go before the effects of nitrogen oxides will be defined with any accuracy, but it is already clear that the first gloomy forecasts, on which the original exhaust standards for 1976 were based, were too pessimistic; it looks as if this part of the exercise will be relaxed. But, then, there is no certainty that the air quality in city centres, those of Los Angeles and Chicago in particular, is linearly related to the numbers of motor cars found there at any time—other sources of pollution are relevant. It is, however, certain that imposing restrictions on the emission of pollutants from cars that travel the wilds of Montana and Wyoming is for the most part entirely irrelevant to the concentration of carbon monoxide and sulphur dioxide, not to mention hydrocarbons, in city centres. One of the fundamental weaknesses of Mr Ruckelshaus's now abortive attempt to clean up the pollution of city centres is that it has been too much concerned with the performance of individual machines and not with an analysis of the whole system by means of which city traffic contributes to city pollution.

The second fundamental aspect of the case which Mr Ruckelshaus has now abandoned is that the new standards were to be applied according to a timetable which is certainly arbitrary and possibly unrealistic as well. It is entirely laudable to seek to clean up the streets of city centres, but it must surely be the case that the cost of

doing so must increase disproportionately with the foreshortening of the time scale. At this point, the automobile manufacturers have had just under two years in which to provide means of removing all but ten per cent of the pollutants in the standard motor car roughly eighteen months from now. Whatever complaints may now be levelled against the automobile manufacturers in Detroit, there can be very little doubt that a period of less than four years is ludicrously short for the provision of what is essentially a radically new kind of automobile engine, with quite different characteristics from those put on the market in the recent past. In the circumstances, it is no wonder that nobody at this stage can put his hand on his heart and say that the catalytic converters in which Detroit has been working will perform satisfactorily and last for the statutory 50,000 miles. And although there has recently been great scorn for the slowness of American manufacturers to follow the Japanese both with the development of rotary engines (to everybody's surprise, now capable of being cleaner than conventional internal combustion engines) and with the development of engines fed at different points with gasoline mixtures of different strength, it tends to be forgotten that Japanese manufacturers have had a head start through their early acquisition of patent rights and that, in any case, these new devices have not yet been applied to vehicles as big as those now used in the United States.

What else might the Administration have done? Of the need that all suppliers of vehicles in the United States should meet the same standards, there is no doubt. At least so long as the cost of making engines which are clean is likely to be greater than the normal, it is unreasonable to expect that manufacturers will off their own bats adopt progressively more stringent standards. If air pollution in city centres is a substantial problem, it is entirely proper that governments should seek to control it by specifying the regulations which it considers must be met. It is not, however, an unpedantic question to ask whether responsibility for satisfying whatever criteria are laid down should rest not with the manufacturers of motor cars but with those who use them. If it were possible to show that air pollution in cities is linearly related to the car population there, it might still be more equitable to require merely that those who choose to drive cars in city centres are responsible for making sure that their vehicles are fitted with catalytic converters or otherwise respond to specified criteria. Individuals would then be able to choose, from a knowledge of their own habits, whether they should buy a clean car or a standard car, recognizing that in the second case they would have to refrain from driving about city streets. It is true that many Americans would regard such a discrimination between vehicles as an assault on the inalienable right of all Americans to drive where they will. But is it not absurd to impose a uniform restriction on everybody simply so as to preserve the illusion that everybody is free?

The truth is that the preservation of air quality in city



centres must in the long run be determined by a judicious combination of specifying criteria which individual vehicles must satisfy when they happen to be in city centres and the restriction of the numbers of vehicles which are able to get there. There is at least a case for thinking that the benefits that might be derived from the hastily devised catalytic converters, dubious in their efficacy as they are, could at least in part have been achieved simply by saddling each city centre motor car with a tax or levy equal to the cost of the extra equipment. Sooner or later, those who use motor cars will be forced to temper their belief in the freedom of travellers to travel where they wish with an awareness of the social cost of travelling in congested places. Certainly there is no doubt that the most economical way of controlling both pollution and congestion in cities must depend on what the economists call an internalization of the externalities—making the motorist bear not merely the costs of operating his vehicle but the costs of what he does to other people.

Under present circumstances, Mr Ruckelshaus and the American Administration as a whole are caught in an awkward dilemma. For one thing, the new cars, whenever they are introduced in 1975 or 1976, will be less efficient and will consume an extra amount of fuel variously estimated to be between 25 per cent and 40 per cent. At a time when the United States is as much concerned with securing its supplies of petroleum for the immediate future as with the quality of the atmosphere in city centres, this is an awkward but significant if unforeseen accompaniment of the emission regulations. That, by itself, might be sufficient reason for a postponement of the regulations *sine die*. Another difficulty is that the United States cannot insist on the rapid implementation of the auto emission regulations without simultaneously increasing the demand for Japanese cars and not the products of Detroit. But is it politically feasible, let alone wise, to incur such an import bill when the balance of payments of the United States with the rest of the world is already adrift and likely to increase negatively in the years ahead still further as imports of petroleum grow? In other words, for all Mr Ruckelshaus's brave words last week, the truth is that the United States cannot afford to implement his regulations. To do so would entail such an increased pace of growth in the trade deficit that the dollar would have to be devalued yet again. The sad truth, in other words, is that the United States has been brought face to face with one of the principal features of any attempt to improve the quality of the environment—that any improvement costs money and that improvements carried out on a crash time table cost still more.

So must the Environmental Protection Agency and Mr Ruckelshaus (while he remains) sit on their hands and hope for the best? Luckily, there are constructive steps that can be taken. First of all, there are potentially great benefits in the proper understanding of the relationship between vehicular traffic and the pollution of city centres and the better understanding of how people may be damaged by specific concentrations of polluting gases. Moreover, such a programme of research could be mounted quickly—parts of it are already well under way—and a year from now enough could be said to make the problem much more tangible than it is at present. Second, there is much that could be done by fiscal means to encourage the production and sale of much smaller

internal combustion engines. It may be true that distances in the United States are, in general, much greater than elsewhere, but city vehicles stand out among those which travel comparatively short distances. So why not identify the characteristics of vehicles which contribute to city pollution—cubic capacity might be one—and then tax accordingly? For the most distant future, however, there are plainly great possibilities in the radical redesign of internal combustion engines with rotary engines and multi-carburettor systems. It has been conventional, in the past few years, to complain that Detroit has neglected these possibilities. It would be much more accurate to say that Detroit has not had time but that the Administration, with its unparalleled access to facilities for research and development in government laboratories, might in the past year or so have sponsored the kind of research that would have allowed Mr Ruckelshaus to know that he was not standing on thin ice and, by judicious licensing of patents, forced Detroit along whatever was eventually considered to be the socially desirable path of innovation. As things have turned out, however, the auto emission regulations will rank with the ban on cyclamates as an illustration of how it is not feasible to conjure up administrative solutions for technological problems by waving a magic wand or even by passing an Act of Congress. To cry for the Moon is permissible only so long as there is a certain scepticism that the product can be delivered.

100 Years Ago



FOR the purpose of more fully carrying out the law of Congress in reference to the propagation of useful food fishes in the rivers and lakes of the United States, the United States Commissioner of Fish and Fisheries made arrangements with Mr. N. W. Clark to hatch out several hundred thousand white fish eggs at his establishment at Clarkston, Michigan, with the special object of transferring them, in due season, to the waters of California. At the proper time, in February last, two hundred thousand eggs were carefully packed and forwarded to California; but, for some unexplained reason, they were nearly all dead on their arrival. In no way discouraged by this experience, the Commissioner directed the shipment of a second lot of two hundred thousand eggs, which arrived in good condition, and the greater number have since hatched out at the State hatching establishment at Clear Lake, into which body of water they will be put at the right time. The feasibility of shipping the eggs of white fish over so great a distance has now been satisfactorily solved, and there will probably be no difficulty in carrying on this work to any desirable extent. Mr. Stone has returned to the East with the view of procuring living black bass, eels, perch, and lobsters, which he will take back to California in a few weeks, in a special car arranged expressly for the purpose. The California Commissioners appear to be fully alive to the interests involved in the multiplication of the food-fishes in their State, and seem disposed to leave no method untried to accomplish this desirable object.

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OLD WORLD

Which Way Forward for Spanish Science?

by our Special Correspondent

SPANISH science is entering a phase of self criticism and reappraisal. In an unprecedented move to help the scientists and the science policy makers in the country, the association of scientists who work for the Spanish Higher Council for Scientific Research (CSIC) called a meeting in Madrid last week to which scientific luminaries from all over Europe, the United States and Japan were invited.

Apart from organizational difficulties, the Spanish problem is one of where best to concentrate its limited resources for research. Traditionally in Spain, research has been carried out within research institutes, which are in most cases divorced from the universities, and it is only in the past decade that pure research within the universities has been accepted.

A close look was taken at the conference of the way in which Japan has developed since the Second World War. Professor Kankuro Kaneshige of the University of Tokyo, and a member of his country's Council of Science and Technology which advises the Prime Minister, freely admitted that Japanese progress had come about through the development of discoveries made in other countries. But he emphasized that it was Japanese scientists who had put considerable effort into turning the knowledge to commercial benefits.

The way in which France organizes its scientific activity was presented by Mr Philippe Richer, assistant to Mr Pierre Aigrain who now holds the position of scientific adviser to the French Prime Minister.

The comparatively inflexible way in which France organizes its research effort contrasted sharply with the methods by which Britain, with no minister and no overall policy for research and development, organizes its research. Sir Brian Flowers stoutly defended the British way—including the customer-contractor principle—and asked the meeting to decide whether the British system is "an untidy mess or a good example of British pragmatism".

But which way should Spain go? Should it follow the lead of Japan, which was universally agreed to be good for a country in the short term, or should it aim at a longer term solution by building up basic research within universities or research institutes? Or, indeed, should the resources available be somehow divided between these two approaches?

The eagerly awaited contribution to the debate by Mr José Lladó, the deputy president of the CSIC, lost some of its impact when the talk had to be read because of his sudden illness. But the audience was highly pleased with the contribution of Mr Federico Mayor, recently appointed acting president of the CSIC who, as well as revealing the philosophy behind his direction of the council, stressed that in future the research institutes and the universities will have to move closer together.

Professor Isidor Rabi, of the University of Columbia, reminded the audience of the meteoric rise of science in the United States in the late 1920s and 1930s—not, he said, because of any

conscious policy decision but because a lack of restriction encouraged scientists to work hard. Professor Philip Abelson, President of the Carnegie Institute and editor of *Science*, outlined a depressing view of the United States scientific scene. But he gave a bouquet to Bell Laboratories and held it out as a model of "scientific openness". But Professor Abelson was highly critical of the massive increase in cancer research funding that President Nixon announced last year. "Most of the money spent in the United States on cancer research will be wasted," he said.

But in spite of national differences in approach to science and different scales of scientific activity, all the representa-

EARTH RESOURCES

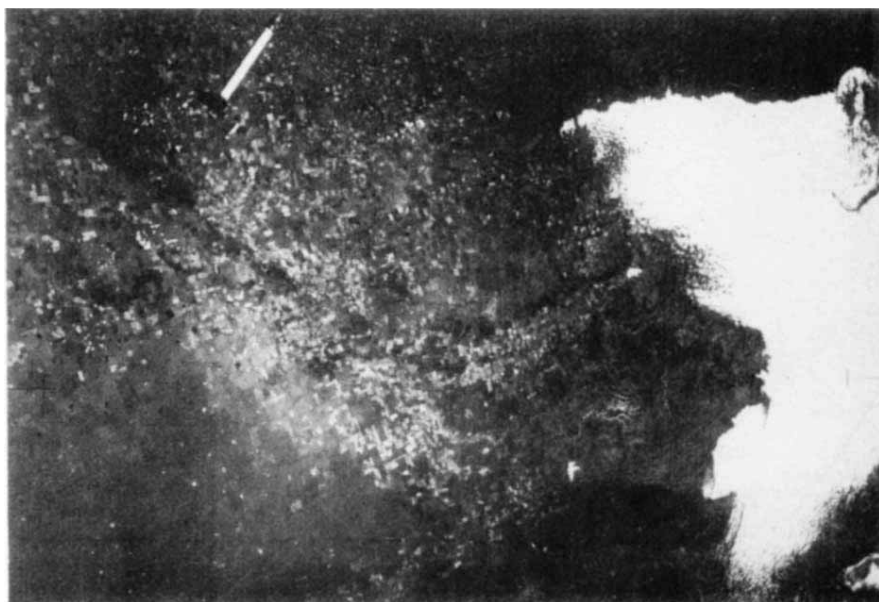
Skylark's Eye View

THIS near infrared photograph of some 300,000 km² of Argentina was taken by a Skylark rocket on its first operational use as an Earth resources survey. The photograph, one of 650 taken during two Skylark flights in late March, shows an area to the east of the Andes in the south of the Cordoba province from a height of 200 km. Cloud to the right of the picture hides the Andean foothills from which a number of rivers flow, and the different tones of the fields indicate the various types of crop that are being cultivated. A town can be seen clearly in the centre of the photograph with roads leading to it. The Skylark's rocket body, which separated from the payload 57 seconds after launch, can be

seen falling away in the top left of the picture.

The survey will reveal land use and provide crop inventories of Argentina's chief agricultural region, and the photographs are currently being analysed by members of the University of Reading's Geography Department and Argentina's Instituto Nacional de Tecnologia Agropecuaria.

The next use of Skylark as an Earth resources rocket will be in northern Sweden this summer when a vegetation survey will be attempted. The chief advantages of using Skylark for Earth resources are said to be cost (launch only costs between £100,000 and £150,000) and the fact that launch can be delayed until cloud conditions are perfect—as happened in the Argentinian survey.



tives when asked were in agreement that Spain must make its own mind up on how to frame its science policy. There was plenty of food for thought for Spanish scientists and science policy makers at this meeting. It now remains to be seen whether it is digestible.

LINEAR MOTORS

We Shall Overcome

A NATIONAL centre to study electrically propelled surface transport is to be established by Imperial College, University of London. This is the reply of Professor Eric Laithwaite and his colleagues at Imperial College to the recent government decision to cancel the tracked hovercraft project (see *Nature*, **241**, 492; 1973).

Professor Laithwaite, appearing before the Select Committee on Science and Technology last week, ridiculed the government's plans to continue research on linear motors through a joint programme of work between the National Research Development Corporation (NRDC) and Hawker Siddeley, with British Rail studying the problems of magnetic suspension.

Hawker Siddeley, said Professor Laithwaite "will be starting from scratch" in this type of work and he cannot understand why the government chose this company in preference to others, in particular GEC or Linear Motors Ltd of Loughborough, which already have expertise in linear motors.

Professor Hugh Ford, also of Imperial College, outlined to the select committee the reasons why it is essential to set up a national centre for this type of work. Britain must maintain its lead in linear motor technology and for this to be done the efforts of the universities and industry must be coordinated. Professor Ford was adamant, however, that the present scheme is not an effort to resuscitate Tracked Hovercraft Limited although an essential part of the plan is to use the test centre which THL has constructed in Earith.

Both Professor Laithwaite and Professor Ford stressed that research into surface transport using linear motors has to be done with a full scale track. As this is already available at Earith it is the natural place to site the proposed centre.

The plan is to fund the proposed centre with money from the research councils, universities and industry. Money will be sought both in Britain and abroad. A permanent staff at Earith of about 30 people is envisaged (Tracked Hovercraft Limited employed more than a hundred). Imperial College will play a large part in running the centre simply because it is recognized that the expertise that the college has in transverse flux motor design and electromagnetic levitation is far more

advanced than that found anywhere else. The centre will be a contract research organization that will, according to Professor Ford, assist the British linear motor and magnetic suspension programmes.

But the select committee could not allow this opportunity to pass without delving further into the government's decision to stop supporting THL. Professor Laithwaite admitted that he was not formally consulted on the closure of Tracked Hovercraft Limited although he modestly admitted that he is the world's leading expert on linear motors. Neither the Department of Trade and Industry, the Department of the Environment or British Rail had asked his advice on this matter although he



Professor Eric Laithwaite.

had discussed it with a representative of the National Research Development Corporation. British Rail, said Professor Laithwaite "does not want to know me", as it is firmly wedded to the idea of steel wheels on steel rails.

British Rail is committed to the Advanced Passenger Train moving along existing track at 150 mph but Professor Elliot of the City University, who also appeared before the select committee, expressed severe reservations about the APT. There are two chief problems, said Professor Elliot. First, it is doubtful whether the track can be maintained to the standard necessary to carry trains at such speeds and, second, it is questionable whether the train will make efficient electrical contact with the live rails at 150 mph.

But Professors Elliot and Laithwaite cast a more serious doubt on British Rail's plans by challenging the basis of BR's decision to base the future of high speed transport in Britain on trains running on existing track, thus avoiding an expensive track building programme. How can the present intensity of slower

speed rail traffic be compatible with the APT, they asked? A train intended to run from Manchester to London in an hour will need a clear run, so where will the freight and other traffic go? The only solution, according to Professors Laithwaite and Elliott, is to build new track for the high speed trains. This removes the chief argument for persisting with British Rail's steel wheel on steel rail policy.

In a carefully phrased set of questions Mr Ted Leadbitter got Professor Laithwaite to relate details of a conversation he had with Dr Ieuan Maddock, chief scientist at the Department of Trade and Industry, after Dr Maddock had been shown a demonstration of the work of the Imperial College group. At the end of what had obviously been an impressive demonstration Professor Laithwaite said that Dr Maddock "clenched his fist and said I wish that I had known all this sooner". The demonstration took place on February 5 of this year. The decision to cancel the Tracked Hovercraft work was reportedly taken in January although it was not made public until February 14.

NUCLEAR STRUCTURE FACILITY

Enquiry Holds Up Work

THE Department of the Environment has called in the scheme to build a nuclear structure facility at Daresbury and a planning enquiry is to be held. This unexpected move by Mr Geoffrey Rippon's department could hold up construction for several months.

Professor Alick Ashmore, Director of Daresbury, the Science Research Council's nuclear physics laboratory, said this week that he was surprised by the department's decision, particularly as Cheshire County Council, within whose area Daresbury falls, had willingly supported the planning application in February. "If the plans had been called in before then," Ashmore said, "I would not have been surprised, but at this stage it is rather unexpected."

Assuming the enquiry allows the SRC's plans to go ahead, Daresbury will have a 20 to 30 million volt electrostatic accelerator by about 1978. The project, which the SRC approved in January, will cost £5 million over four and a half years, and will provide Britain's nuclear structure physicists with a low energy accelerator that they have waited ten years for already (see *Nature*, **237**, 192; 1973).

Professor Ashmore said that having the plans called in is bound to produce a delay. "We can carry on with general research and development on higher voltages," he said, "but we can't start to build." Work is also being held up by the Department of Education and Science, which has still to give the SRC permission to spend the £5 million.

NEW WORLD

Auto-Emission Standards Postponed

by our Washington Correspondent

MR WILLIAM D. RUCKELSHAUS, administrator of the Environmental Protection Agency, last week sacrificed the Federal Government's plans to clean up city air for the financial wellbeing of the corporate giants of Detroit. That, at least, was how spokesmen for several environmentalist organizations greeted his decision to grant car makers an extra year to produce vehicles which meet stringent standards for exhaust emissions. But spokesmen for the corporate giants take a different view of Mr Ruckelshaus. They have accused him of putting thousands of jobs in jeopardy by drawing up a new set of emissions standards which are still impossible to meet on time.

By any measure, the decision which Mr Ruckelshaus was forced to take last week was a vexed one. In short, he had to decide whether or not car manufacturers are capable of making 1975 model cars which emit 90 per cent less carbon monoxide and hydrocarbons than those produced in 1970. If he decides that they cannot, he is allowed by law to grant them one year's grace and to set fresh standards for 1975.

At stake, Mr Ruckelshaus pointed out, are billions of dollars, hundreds of thousands of jobs, the single most important segment of the US economy, the largest aggregate manmade contributor to air pollution and the ambivalence of the American public's intense drive for healthy air and apparently insatiable appetite for fast, efficient and convenient automobiles. He might have added to the list the future of the Clean Air Act.

A year ago Ruckelshaus decided, on the basis of a lengthy set of public hearings, that the technology for meeting the emissions standards would be available by 1975, and he denied the car makers' request for a year's extension. But he was forced by an Appeals Court ruling in February to re-examine his decision and after yet another set of hearings he backed down and granted the request. But no champagne corks were popping in Detroit in celebration, because Ruckelshaus has set stringent emissions control standards for California in 1975—which will force manufacturers to fit emission controls to cars sold there—and standards for the rest of the United States which go half way towards the original 1975 standards.

What caused the EPA administrator to change his mind? The most important factor was the Appeals Court ruling which allowed him to bring considera-

tions of social and economic impact on the automobile industry into his decision. Another was a report drawn up by a committee of the National Academy of Sciences which suggested that if the 1975 standards were adhered to, the manufacturers would be forced to adopt what the committee considered to be bad technology, and a third factor was that several Japanese car makers have stolen a march on their American competitors by bringing out an engine which is capable of meeting the standards and which looks a better bet in the long run for ensuring that city air is cleaned up.

The essence of Ruckelshaus's decision involves the question of whether or not oxidation catalysts fitted to car exhausts will work and whether or not car makers can install them in time. US automobile manufacturers opted at an early stage to keep the conventional piston engine and to try to clean up the exhausts with platinum catalysts, but some Japanese manufacturers took a more radical approach which looks like paying off. They intend to fit their cars with stratified charge and Wankel engines, and in fact Honda and Toyo Kogyo have already produced test vehicles which meet the 1975 standards with ease.

The committee of the National Academy of Sciences reported in February that the catalyst would probably work but said that it is less reliable, heavier on fuel and generally a worse option than the stratified charge engine. Ruckelshaus was therefore faced with the following choices. Either he could force all US manufacturers to comply with the 1975 standards on time, in which case they would have no alternative to using catalysts, or he could relax the standard in the hope that car makers would use the extra year to switch to other engine designs, but that is generally considered unlikely. A third alternative, and the one he eventually chose, would be to force manufacturers to fit catalysts on some of their cars in 1975 and to ensure their widespread use in 1976.

His decision will force manufacturers to fit catalysts to all 1975 cars sold in California—which represents about 10 per cent of the US market—and to all their 1976 models. Ruckelshaus took that course because he believes that "the oxidation catalyst is workable and the catalyst is the technology which must be used if the statutory standards are to be met by 1975 or 1976". In other words, since the US manufacturers put all their eggs in one basket by opting for cata-

lysts rather than by engine redesign, their only hope of meeting the standards—even if they are deferred for a year—will be with catalysts.

By forcing manufacturers to phase in catalysts over two years rather than insisting that they be used on all 1975 models, Ruckelshaus said that he is attempting to avoid widespread social and economic disruption in the car industry. But he is also concerned to prevent the Clean Air Act from being torpedoed from Detroit.

If Ruckelshaus had stuck to the 1975 deadline, it would be a foregone conclusion that the car makers would quickly come running to Washington to see their congressmen and senators in an attempt to alter the Clean Air Act and their potential for blackmail would be immense. They would simply say that their production lines would not be able to fit catalysts in the time allowed, that production of cars would have to be curtailed, hundreds of thousands of people would be laid off and Detroit would be turned into an economic disaster area. Backed by a nation-wide advertising campaign, such arguments would swiftly bring Congress round to Detroit's way of thinking.

In steering a middle course between Detroit and its critics, Ruckelshaus may have succeeded in blunting some of the car makers' arguments for repealing or cutting a wedge into the Clean Air Act, and the EPA hopes to show that the economic dislocation caused by the two-phase introduction of catalysts will not be great.

But Detroit is already girding its loins for battle and it will wage war on two fronts. First, it will argue that the interim standards that Ruckelshaus has set for outside California cannot be met without the use of catalysts, and second, it will attack another provision of the Clean Air Act, namely that emissions of oxides of nitrogen must be reduced by 90 per cent by 1976. To help in its campaign, the manufacturers have already engaged the help of their bedfellows in the oil industry whose interest stems from the fact that exhaust catalysts will require unleaded gasoline which the oil companies do not want to provide.

A taste of the possible campaign was given last month when full page advertisements were taken out by car makers pointing out the costs of emissions controls and the fuel penalties that will fall on the owner. An advertisement from the Mobil Oil Company, for example, called the Clean Air Act "a

66 billion dollar mistake". Not to be outdone, however, the Sierra Club responded with an advertisement urging Ruckelshaus to hold fast on the 1975 deadline.

As for the manufacturers' first point, Ruckelshaus stated last week that "the technological information available to me indicates that few catalysts will be required to meet this standard". But the manufacturers chorused that their information leads them to a different conclusion, and they hinted that they may take their case either to the courts or to Congress.

But as far as the standards relating to oxides of nitrogen are concerned, they may be on firmer ground and in any case they are likely to have the backing of the EPA. In short, the EPA has set a limit of 100 micrograms of nitric oxide per cubic metre of air as the maximum level to protect health and it originally estimated that the limit is exceeded in 45 regions throughout the United States. But it has since re-evaluated the concentrations using more precise techniques and will soon disclose that the level is exceeded in only two regions—Los Angeles and Chicago. Ruckelshaus in fact said last week that "our assessment of the health risk associated with (oxides of nitrogen) no longer supports the 90 per cent reduction standard and this should be reviewed quickly and, if our analysis is correct, the standard should be changed".

The automobile companies would be delighted if Congress accepts the need to change the standards because they have not yet found a reliable method for reducing emission of oxides of nitrogen. So far, US companies are concentrating on a dual catalyst approach but the NAS committee said in its report that no system has yet been found to be reliable and that the dual catalyst has a huge fuel penalty, increasing gasoline consumption by up to about 25 per cent. The stratified charge engine developed by Honda seems capable of meeting the standard for oxides of nitrogen as well as those for hydrocarbons and carbon monoxide, however.

The reason why the EPA is anxious to protect the Clean Air Act from being butchered is that it involves regulation of many sources of pollution in addition to automobiles and that changes in one area will affect others. The act requires the EPA to set ambient air quality standards nationwide and the states are empowered to meet the standards according to a specified timetable based on the emissions standards now established. But if the car makers are given more time to control exhaust emissions, many of the states' plans for meeting the air quality standards could be thrown badly out of kilter.

In the final analysis, however, the US car makers have only themselves to

blame if they end up using inferior technology to curb exhaust emissions. They began to work seriously towards pollution control only when the Clean Air Act put a pistol to their heads and, unless they can now bully Congress into giving them a second chance, they may have to pay for their mistakes on the market place when they compete with the Japanese companies.

ENERGY RESEARCH

Rumblings from Congress

by our Washington Correspondent

ALTHOUGH statements, reports and recommendations on the so-called energy crisis are by now two a penny, a report published last week by a special congressional task force merits careful reading. It suggests that spending by government and industry on research and development on new sources of energy has been unbalanced and insufficient, and calls for at least an additional \$1,000 million a year. The report is important because the task force which produced it has recently been promoted into a full subcommittee of the House Committee on Science and Astronautics, and as such will have a strong voice in shaping legislation dealing with energy research. It is also a useful yardstick by which to measure President Nixon's long overdue and eagerly awaited statement on energy policy, originally promised for February and now expected to be unveiled this week.

In short, the task force, which met under the chairmanship of Mike McCormack, an energetic young congressman from Washington, took a careful look at a report prepared for President Kennedy in 1964 by an inter-agency committee, and concluded that most of its recommendations "are still valid today—valid because so little action has been taken to implement them". The task force also recommends that an energy policy group should be established in the White House and that all the energy programmes of the Federal Government should be centralized in a single agency.

The task force does not say exactly how the extra \$1,000 million should be spent, or how it should be divided between the Federal Government and industry. But it does suggest that past spending has been biased towards nuclear energy, and singles out seven priority areas which hold great promise and deserve more money. They are basic research—for example, the National Science Foundation's RANN programme—materials science, solar energy, geothermal energy, breeder reactors, coal gasification and liquefaction and controlled thermonuclear fusion. Of these, only the breeder reactor has been given high priority by the Admini-

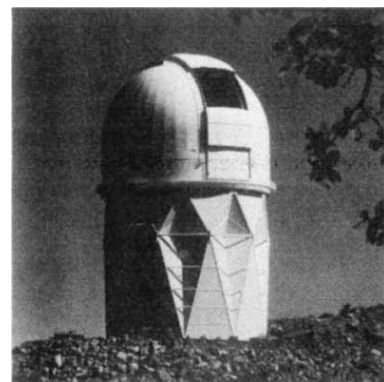
stration, and then the money has been put into the liquid metal fast breeder almost to the exclusion of other designs.

The suggestion that an extra \$1,000 million should be spent on energy research and development is based on the task force's belief that 10 per cent of the total national research and development effort should be devoted to energy. Given the technologically intensive nature of the energy industries, such an expenditure is not unreasonable, the task force states, since total outlays on research and development in the United States are running at a little under \$30,000 million. Such a commitment would require about \$3,000 million to be spent on energy research. But only some \$2,000 million is expected to be spent next year, and of that about \$750 million will come from the government.

Viewed in conjunction with the energy research and development programme proposed by Senator Henry Jackson and several of his colleagues (see *Nature*, **242**, 224; 1973), the task force's proposals indicate which way the wind is blowing on Capital Hill. Jackson's bill calls for expenditures by the Federal Government of \$2,000 million a year over the next ten years on non-nuclear energy research.

Meanwhile, President Nixon's budget for next year calls for only \$772 million to be spent on energy research and development, and in view of the Administration's present cost-conscious mood, the forthcoming energy message is not expected to add much more money to the budget request. Funding for energy research is thus likely to be yet another bone of contention between the White House and Congress.

Erratum



THE photograph that inadvertently appeared on page 364 of this volume of *Nature* was, of course, of Britain's Goonhilly 3, a £2.25 million satellite communication station which was opened last July. The 158-inch Mayall Telescope that should have appeared is pictured above.

NEWS AND VIEWS

The Screw Worm Strikes Back

THE eradication of the New World screw worm, *Cochliomyia hominivorax*, first from Curaçao and then from the continental United States through the release of factory-produced sterile flies can be counted a scientific and an economic success both from the novelty and acceptability of the method and from the rapidity with which the losses suffered by the American livestock industry were overcome.

The danger of re-invasion from uncontrolled Mexico, however, required a permanent, continuous "barrier" of releases extending some 250 miles in width along the 1,500 miles of the USA-Mexico border. During the operation of the scheme practical imperfections in this barrier became evident. These stemmed from (a) the limited capacity of the single, permanent sterile fly-factory at Mission, Texas (producing approximately 100 million sterile flies of both sexes per week); (b) the flight range of the female screw worm fly (up to 180 miles) (B. G. Hightower, in *Insect Ecology and the Sterile-male Technique* (Proc. Panel, Vienna, 1967), 25 (IAEA, Vienna, 1969)); and (c) the length of the USA-Mexico border.

International and domestic cooperation in monitoring screw worm activity in the barrier area with a view to gauging the density and spatial pattern of aircraft release of sterile flies has been used to offset these imperfections, and it was initially successful. Recently, however, there has been a major setback, judging from the published figures in the weekly *Cooperative Economic Insect Report*

published by the United States Department of Agriculture (see Fig. 1 and Table 1).

Table 1 Confirmed Cases of Screw Worm, USA and Mexico

	1962 *	1969 †	1970 †	1971	1972 ‡
United States	50,000	219	153	473	92,192
Mexico §	—	—	4,634	8,811	27,467

Data summarized from the *Cooperative Economic Insect Report*, US Department of Agriculture.

* Estimate.

† "Lowest 2 years ever."

‡ December 18, 1971, to November 18, 1972, except May 14 to 27.

§ As most of these cases were reported from the "barrier zone", the totals for Mexico are likely to be gross underestimates.

In 1962, after a successful eradication scheme in Florida, the United States south-west project was launched. By 1966 only small numbers of cases of livestock infection could be detected, with an all-time minimum of 153 in 1970. The rise to 473 in 1971 was hardly a portent of things to come unless a discerning eye could attach some significance to the fact that more than 50 per cent of the cases were registered in September and October rather than earlier in the year. In 1972 a cumulative total of 500 was surpassed in the week of April 17 with the greater part of the spring still to come. Ominously the weekly confirmed cases rose to 609 during the second week of May and a peak of 5,013 was reached at the end of June. During July the tide seemed to recede, but by the end of August the 5,000 mark was almost touched again. A slow decline, probably reflecting the season, followed, but more than 3,000 per week were being recorded by the end of October. As 92,192 cases could be counted from December 18, 1971, to November 18, 1972, the estimate for 1962 (50,000) was surpassed, though it should be pointed out that nowadays in the United States case finding and reporting are more efficient than in the past.

At present it is not known exactly why the screw worm returned. A possibility is that there has been a behavioural selection for assortative mating which has diminished the efficiency of the factory-produced sterile males. In other words, there may have been an increase in the likelihood of a wild fly being mated by a wild fly rather than a sterile one, given an equal choice. If this is the explanation one wonders whether the regular incorporation of fresh, wild material into the sterile-fly factory could increase the efficiency of the releases. Perhaps the selection inherent in colonization and mass rearing also affects the mating behaviour independently of any direct effect of the sterilization operation.

In response to this return of the screw worm the number of sterile flies released was doubled to about 200 million (half of them males) per week during 1972, but this increase was obviously insufficient because the reported

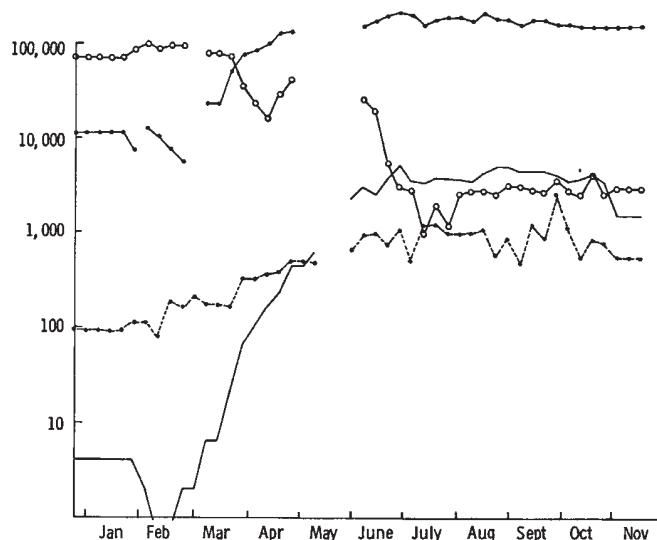


Fig. 1 Cases of screw worm and releases of sterile flies, 1972, per week, United States and Mexico (data from the *Cooperative Economic Insect Report*, US Department of Agriculture). ●—●, Releases (thousands) in the United States; ○—○, releases (thousands) in Mexico; —, cases in the United States; — — —, cases in Mexico.

number of screw worm cases in the United States followed a familiar seasonal pattern. Concomitantly, as the pest spread back into the originally infected (receptive) areas of the United States, both the relative and the absolute number of sterile flies released over Mexico declined. On October 29, 1972, a press release in Mexico City specified that following an agreement with the United States government a 5-year nationwide screw worm eradication effort would start during November and would involve the building of a sterile-fly factory at Salina Cruz in the Tehuantepec Isthmus with a capacity of 300 million sterile males (probably weekly). This suggests that the barrier would eventually be in the waist of Mexico, as proposed by Hightower and Graham (in *Control of Livestock Insect Pests by the Sterile-male Technique* (Proc. Panel, Vienna, 1967), 51 (IAEA, Vienna, 1968)) (see Fig. 2) though it has already been suggested by Bushland (in *Sterility Principle for Insect Control or Eradication* (Proc. Symp., Athens, 1970), 3 (IAEA, Vienna, 1971)) that a better, more logical place would be in Panama and Costa Rica, where Central America narrows to a width of less than 100 miles for hundreds of miles.

Besides the barrier problem there is the formidable task of re-eradicating the screw worm from the United States. Even with the help of the weather, a principal factor in determining the size of the overwintering area, which is much smaller in comparison with the summer receptive area, it seems doubtful whether the screw worm could be quickly beaten back to the 1970 level with the present material, procedures and logistics.

Assuming, optimistically, that increased numbers of factory-produced sterile flies are all that is needed, hope comes squarely to rest on the projected Mexican, Salina Cruz plant (if the Mission, Texas, plant is not expanded or duplicated elsewhere in the United States). Its sterile flies could then supplement the releases in the United States or could be used independently, working northwards, after securing the Tehuantepec barrier. The eradication of the fly from Mexico, however, also presents potential difficulties in the way of: (a) mountainous terrain and difficult communications; (b) a relatively very small area subject to winter conditions inimical to screw worm survival; (c) higher density, compared with the United States, of livestock in general and in particular of sheep and goats (considered better hosts for the fly);

(d) absence of large livestock ranches with unified management; and (e) constant livestock movement towards the central consuming centres from and through the Tehuantepec barrier. Furthermore, the tropical moist conditions of some of the livestock areas of Mexico in the Tehuantepec barrier or further to the north may well require different procedures and/or release ratios in excess of those which have been found sufficient in the United States. At present, however, it is not at all certain whether increases in the weekly figures of releases of factory-produced sterile flies in their present form are the answer required. Until this matter is settled by adequate research or by a successful re-eradication from the United States it could be wasteful to attack the tropical habitats of the fly. It is now known that both Mexico and the United States have set aside \$40 million to be spent on the eradication of the screw worm during the next 5 years and on the establishment of a barrier zone.

C. B. C.
D. M. B.

Knack or Knowledge?

THE transistor was invented twenty-five years ago and the stir created by its anniversary has now abated a little. Having heard in full about the benefits which that invention has provided (see, for example, *Nature*, **242**, 91; 1973), it is perhaps time to consider a weakness which appears in the successors of Bardeen, Brattain and Shockley—one which they studiously avoided—namely the tendency to use knack rather than knowledge to achieve difficult goals in solid state technology. Unfortunately many impatient men in the massive solid state component industry try to run before they can walk, with the results that usually attend such overconfidence.

The transistor has endowed tremendous scope for performing electronic functions (for example switching, which permits binary logic, and amplifying, which makes possible many other forms of signal processing). The chief advantage over the valve is the ability to perform those electronic functions within very small volumes of solid. For example, a switch may occur roughly $100 \mu\text{m}^3$, which should be compared with the volume of a pinhead—many millions of μm^3 . The smallest conceivable valve device may be several times larger again. As a result one can now, with some ease, put a minicomputer on a postage stamp (the 'Intel' silicon microprocessor integrated circuit is actually about 1 cm on a side).

For several reasons, however, semiconductor technologists are still trying to get the minicomputer, metaphorically, onto the pinhead. This is not, of course, only showmanship. If this density of electronic functions can be achieved, solid state shift registers may replace magnetic tape recorders and solid state random access memories will have such large bit densities that, to quote a punning headline "Magnetic Cores Will Be . . . Just a Memory". The problem lies in the fact that technological skills are not advanced enough to achieve the densities of function which these commercial ambitions demand. Knowledge of the processes which go into making high density integrated circuits is not accurate enough. Integrated circuits may be mass produced but their preparation involves several intrinsically complex processes, such as impurity

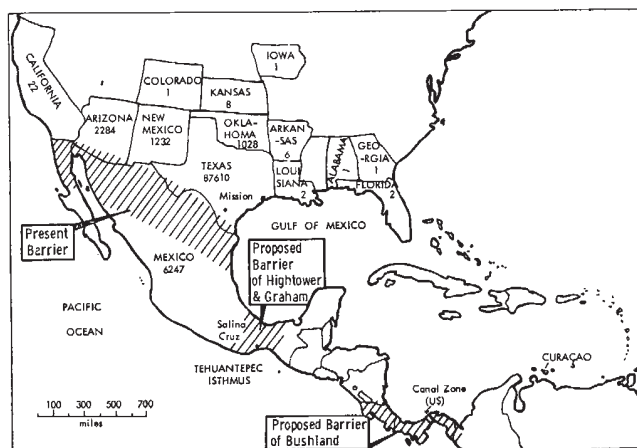


Fig. 2 Cases of screw worm, 1972, and barriers (data from the *Cooperative Economic Insect Report*, US Department of Agriculture).

diffusion in the solid, thermal oxidation of surfaces, pyrolysis of gases and evaporation of compounds. On the whole, industrial scientists have limited themselves to characterizing the broad features of these processes, for example the temperature dependence of diffusion and the dependence of resistivity on impurity concentration. The intense competitiveness of the semiconductor industry has generally made the same scientists stop short of the more fundamental questions of mechanism. For example, it is not well established whether diffusion of dopants occurs by way of vacancies or by dislocation climb (Peart and Newman, Institute of Physics Conference Series No. 6, 170; 1973); and knowledge of the structure of the thermally-grown and deposited dielectric layers, used both as diffusion barriers and electronic barriers in planar silicon technology, is rudimentary in the extreme (see Revesz, *J. Non-Cryst. Solids*, **11**, 309; 1973).

An optimist would say that such fundamental knowledge is not necessary in order to achieve an industrial production process; the right collection of recipes for temperature, time, input materials and so on should permit a limitless number of batches of an established device to be made with good repeatability and high reliability of product. In practice, this repeatability is achieved, if at all, by dint of long struggles and empirical means and the industry suffers seriously from this lack of predictability. It is perfectly common for some uncontrolled variable to change and for the yield of a production run to fall far below a commercially acceptable level. Everybody in the industry has a story of a production line which "lost the knack" for a few weeks. This applies to processes other than transistor preparation, of course, and other industries may have helpful lessons to teach. The situation in the solid state industry simply seems to be that skills are being stretched too far; that in the manipulation of solid state phenomena to the dimensional accuracy demanded today much better predictive formulae are needed to foresee the influence of, say, slight changes in starting materials on the dimensions and electronic transport properties of the final melange of atoms which constitutes the desired electronic function. The director of the research laboratory of an electronic company recently said publicly that knowledge of the solid state phenomena used, compared with what one needs to know, is "pathetic" (*Nature*, *loc. cit.*). How, then, does one keep knowledge of the physics of processing matched to the demands? The same speaker ruefully admitted a gap in the research machinery and an accompanying sense of frustration in trying to fill that gap. He felt that the traditional venue for accumulating fundamental knowledge, the university physical laboratory, was not, as constituted now, well matched to the task in the case of the relevant solid state phenomena. On the other hand, he admitted the common difficulty of persuading semiconductor manufacturers to support the work of their own laboratories.

At this stage, one should examine several general features of the problem. First, the research goals are of a type often termed "underlying research", although the problem is perhaps more acute than many tackled under this heading. Second, the problem at present chiefly concerns silicon device technology; certainly all facets of the problem are expressed in this global industrial common factor. More is known about this semiconductor than any other and still the problems obtain; thus, what applies to this material will probably apply to others.

Third, although the problem affects nearly all manufacturers of semiconductor devices, their nearness to the problem may, in fact, give them the wrong perspective on how to treat it as a whole. Industrial laboratories are usually better at developing improved designs or new principles for devices (a more glamorous and profitable business than cleaning up behind themselves) and hence may not see that filling certain gaps systematically is "for their own good". The extent of the physical problems is so wide that at least a national scale is required for their investigation. Thus, the venue of such research should lie between the universities and government establishments, with national coordination. The national need which will most directly benefit from the research will be trade, although defence will also be a principal beneficiary. The benefits may be greater than for most underlying research.

In considering the particular failings of the university in this context, it is clear that the carrying out of research through the medium of graduate students is not well matched to the rigorous requirements of purity and accuracy in research on solid state processing. Continuity in skills and immense care in control of process conditions have been found to be the only answer to repeatability of results in this field. The necessary continuity could perhaps be provided within a university by the provision of a permanent staff more than usually committed to this continuity. This is the intent, it seems, of the several Materials Science Institutes set up in the United States in the 1960s. The disciplines needed are physics, chemistry, metallurgy and general engineering skill. Some departments of electronic engineering, in fact, already pursue approximately the right combination of topics. Government research laboratories have the right degree of stability, but the trend is now towards the project approach. Except, perhaps, in the laboratories interested in standards and metrology, the acceptance of an appeal to a general, underlying improvement may not be easy to find. It is significant that the chief establishments for electronic materials research are under the control of the Ministry of Defence and have well defined but narrow objectives.

As to finding money for such work, the usual arguments for government support of "high technology" apply here, probably more forcibly than usual. It might even be argued that institutes developing an understanding of the physics of processing would be likely to pay for themselves directly in licences and indirectly in the benefit accruing to the nation concerned in terms of efficiency—money saved by the avoidance of setbacks and of waste—and in terms of national trade improvement as semiconductor products become more competitive in the international market. It could be objected that the British government already supports such work in such programmes as the Component and Valve Development and Computer Technology Project. As the titles of these activities suggest, however, each programme is essentially looking for techniques to produce devices of higher performance, rather than searching for the principles behind consistency of performance; moreover, the ultimate uses of devices developed are well defined and the required generality of scope and long term commitment may be missing. What is needed is a general improvement in the accuracy achievable in solid state processing. Cookery is not enough, and knowledge is economically better than knack. A. G. H.-S.

MEMBRANES

Argument

from our Molecular Biology Correspondent A SYSTEM that has done service as an archetype for the structure of biological membranes is the retinal rod outer segment. A feature that especially commends it for such a role is that it contains only one protein in large quantity. After some early skirmishing the X-ray practitioners appeared to reach general agreement about the electron density profile across the bilayer, and more particularly in the conclusion that the electron densities, given the proportions of rhodopsin and phospholipid in the membrane, could not be made to add up for any model embodying protein totally submerged in the bilayer, or plating the surfaces, and were best compatible with globules floating, only partly immersed, in the membrane fluid.

Last year Dratz and his colleagues (*Nature New Biology*, 237, 99; 1972) attempted to shatter this tranquil accord by arguments based on chemical labelling experiments in intact rod outer segment membranes compared with the isolated rhodopsin. In this they seem to have been wrecked on a reef that has accounted for many a good protein chemist in its time, and now familiar to most, namely that the reactivity of side chains in proteins is controlled by a variety of factors besides mere steric accessibility. The plan was to use a macroscopic labelling reagent, which would be unable to enter the bilayer, and could therefore modify only groups exposed to the aqueous exterior. The reagent in question was fluorescein isothiocyanate adsorbed on a colloidal particle, which was found to react abundantly with rhodopsin, free or in membranes that had been broken up with detergent or a phospholipase, but not in the intact or even sonicated membrane. From this they boldly inferred that the rhodopsin *in situ* must be totally buried within the bilayer.

Vanderkooi in this week's issue of *Nature New Biology* (242, 212; 1973) now points out that this will not wash. Besides the evidence of the phalanx of X-ray crystallographers, he draws attention to the likely role of environmental factors in determining the reactivity of the reactive lysine, tyrosine and cysteine side chains of the protein. It is known that rhodopsin reacts *in situ* with aldehydes, and with thiol reagents: three of the latter, all polar iodoacetamide derivatives, react with one of the two reactive thiols of the protein. There is, moreover, the recent work of Blasie, which purports to demonstrate steric changes in the membrane within a pH range in which only charged groups on the protein could change their ionization states.

In their rejoinder Dratz and Schwartz (*ibid.*, 212) seem more concerned with repudiating Vanderkooi's arguments than defending their own position, which they seem in any case to have stealthily evacuated in the interim. The aldehydes, they say, can enter the lipid phase, and react with the protein side chains that they find there. As to the thiol reagents (work reported last year by Wu and Stryer) the results, they assert, prove nothing: two of the reagents have a sufficiently wide separation between the non-polar reactive centre and the polar carrier group that the reaction could occur under the bilayer surface; the third reagent, they believe, might actually be lipid-soluble. Blasie's evidence they counter by questioning whether he has correctly identified an X-ray reflexion that he uses to measure the inter-protein spacing. They have also, they say, calculated Fourier syntheses for the various possible models for the membrane, and whereas some fit the observed electron density profiles in some regards, they fall short in others. This, of course, is not unfamiliar to the crystallographers who have been toiling in the field for some years, and who have recently stressed particularly the complications arising from the structural disorder that is thought to be present. Dratz and Schwartz then come to the point: work of their own and of two other groups, using specific labelling methods in results as yet only available as published abstracts, shows that part of the rhodopsin is indeed exposed to the aqueous medium: the

view that the protein floats partly immersed in the bilayer therefore remains unassailed, and the earlier labelling results were, as one suspected, writ on water.

Also in *Nature New Biology* this week (*ibid.*, 213) is a sequel by Phillips and Morrison to their earlier work on labelling of membrane proteins by a reagent too large to penetrate the bilayer. This is nothing less than a protein, lactoperoxidase, which catalyses the iodination of tyrosine residues. In the red cell, only two proteins are labelled from the outside by this means, one of them the principal glycoprotein, which bears surface receptors for such ligands as plant lectins. As with cells in tissue culture, limited exposure to trypsin results in increased agglutination in the presence of such species, and it has been variously argued that the trypsin treatment leads to exposure of new binding sites, or to increased freedom of the receptor protein to form strongly binding clusters in the membrane. Phillips and Morrison now find that trypsin releases glycopeptides, including one that contains the tyrosines iodinated by lactoperoxidase. In spite of the removal of this reactive residue, the trypsin-treated cells are able to incorporate ten times more label than the untreated, exclusively, moreover, in the same two proteins. These results can be most simply interpreted in terms of the obstruction of much of the surface protein by a fur of carbohydrate, which might well turn out to be a widespread structural feature in membranes.

EB Virus Superinfection of Human Lymphocytes

LINES of human lymphoid cells which multiply indefinitely in culture have, irrespective of their origin, proved to contain Epstein-Barr (EB) virus DNA and EB virus has been implicated in the aetiology of Burkitt's lymphoma and infectious mononucleosis. Cells of many of these lymphoid lines can be superinfected with EB virus even though they contain EB virus DNA.

In *Nature New Biology* next Wednesday (April 25) Adams and Klein report an analysis of the response to superinfection with EB virus of cells of eleven human lymphoid lines, six derived from Burkitt lymphoma tissue and five derived from patients with infectious mono-

nucleosis, a patient with leukaemia and from healthy persons. Their findings lead them to the interesting conclusion that either lymphocytes from Burkitt lymphoma tissue differ in type from non-Burkitt lymphocytes, or different subtypes of EB virus are present in the Burkitt and non-Burkitt cells, or the mechanism of transformation of the two classes of cells differs.

Cells of all the lines studied adsorbed EB virus, and one particular stock of this virus proved to be unusually active in inducing viral early antigen. Adams and Klein therefore used this stock to measure the dose response relationship of the Burkitt and non-Burkitt cells. Cells of eight of the eleven lines fell into one of two classes when their dose response curve for the induction of viral early antigen was measured, the non-Burkitt cells having a linear dose response whereas the Burkitt cells had something approaching an exponential dose response. Moreover, three lines, two Burkitt and one non-Burkitt, responded with the production of early antigen much more strongly than the other eight lines.

Correction

THE article by Riddle and Carbon referred to in a note, "Frameshift Suppressor Transfer RNA", in last week's issue (*Nature*, 242, 436; 1973), will be published in *Nature New Biology* for April 25 and not April 18 as stated.

CHALLENGER SOCIETY

Newcastle Meeting

from a Correspondent

THE opening theme of the joint meeting of the Challenger Society and representatives of marine laboratories, held in the University of Newcastle upon Tyne on March 27 and 28, was the gross estuarine pollution of the north-east coast of England. It is an unhappy commentary that a similar theme characterized a meeting of the Challenger Society at the Dove Marine Laboratory exactly fifty years ago.

Dr J. S. Gray (University of Leeds) has been monitoring Seal Sands in the Tees estuary, an area once indeed sandy and harbouring seals, but now a mud-flat. Nevertheless, in spite of heavy industrial pollution the meiofaunal density of the intertidal sediment here averages 2.5 million organisms per square metre. Dr Gray has found that the species diversity of the annelids of the area is reduced in certain limited stretches; it seems likely that pollution here may reach a peak, yet he is not satisfied that this reduction in diversity can be categorically related to pollution.

Dr A. James (University of Newcastle upon Tyne) described the effects of directing polluting discharges into the Tyne estuary, chiefly in terms of the dissolved oxygen regime, but some mention was also made of intestinal bacteria and nutrient budgets. He showed the results of a one-dimensional model which has been developed to help predict future levels of organic matter, bacteria, nutrients and oxygen.

Dr P. G. Moore (University Marine Biological Station, Millport), taking a more generalized view of environmental quality in the sea, also considered the possibility of using ecological diversity values as indices of pollution. He looked particularly at diversity indices biased towards the equitability component. He has found that although there are problems concerned with varying key species and with the choice of animal size and taxa, the principal difficulty lies not in the measurement of diversity but in its interpretation, so that its use as an indicator of pollution, while possible, requires caution.

Dr R. Johnston (DAFS Marine Laboratory, Aberdeen) returned to specific cases and outlined the problems involved in predicting the effects of a proposed new pulp mill on the chemistry of the waters of two Scottish sea lochs, Linnhe and Eil. A decision whether or not to permit a discharge from the mill into the lochs was required in 1961 although the actual discharge would not begin until 1966. The complexities of the hydrographic situation were unravelled and future changes in dissolved oxygen and bio-

logical oxygen demand (BOD) were estimated and found acceptable. Once the mill was working subsequent routine observation enabled the minor reduction in dissolved oxygen and the minor increase in BOD in the water to be correlated with the BOD and suspended solids input of the mill.

The plankton session was opened by Dr F. Evans (University of Newcastle upon Tyne) who spoke of the long history of plankton sampling in Northumberland coastal waters, extending as far back as 1860 although in a much broken series. The Northumberland plankton does not, he said, derive at all from populations to the south of the county but consists of species constantly found locally, supplemented intermittently by immigrants from the north. This accords with the predominantly southward flow of wind-induced currents close inshore, but it is a matter for remark that species well known only 50 miles to the south are not brought northwards into the region during times of contrary winds and accompanying northerly flow.

A "Challenger" plankton session would seem incomplete without a contribution from Edinburgh, and the demand was met by Dr R. Williams

(Institute for Marine Environmental Research) who gave an account of the extensive sampling programme at Ocean Weather Station "India". The equipment used includes sophisticated sensors of the physical environment combined with a plankton net which incorporates the Hardy-Longhurst cod-end for controlled depth sampling.

Dr M. Shearer (University of Newcastle upon Tyne) dealt with a planktonic group—the hyperiid amphipods of the North Sea—which was previously inadequately known. Of the three North Sea genera, *Hyperia*, *Hyperoche* and *Parathemisto*, he dwelt more especially on the last. His taxonomic studies show conclusively that the large, oceanic *P. gaudichaudi* and the smaller, neritic *P. gracilipes* are in fact the same species; furthermore, laboratory rearing of the young of both forms produces animals which are indistinguishable from each other provided environmental factors are equal.

The plankton session was concluded by Dr S. J. Lockwood (MAFF Fisheries Laboratory, Lowestoft) who reviewed the replenishment of the almost self-contained stock of plaice off the Yorkshire coast. He has found that eggs are spawned some 10 to 20 miles off the

Extraction of Gonadotrophin Receptors

RECEPTORS can be defined as components of a tissue which specifically react with a drug or hormone and which are essential for the expression of the biological activity of the compound. In endocrinology, interest in receptors was renewed about 1960 when it was postulated that specific proteins in tissues of the reproductive tract interact with the sex hormones and are responsible for their uptake by the tissues. Presence of the receptors also explained the specificity of the action of the sex hormones on reproductive tissues. The sex hormones, however, are known to affect metabolic reactions in almost all tissues and presumably therefore proteins which interact with the hormones and which could be called receptors should be present in other tissues as well as those of the reproductive tract. The meaning of "receptor" is becoming very diffuse and a clearer definition of the word is necessary. Receptors which interact with polypeptide hormones have also been shown to be present in tissues; these receptors seem to be membrane-bound and hence their isolation presents difficulties.

In next Wednesday's *Nature New Biology* (April 25) Dufau and Catt describe a procedure for the extraction of gonadotrophin receptors from the interstitial cells of rat testes. After subcellular particles were isolated by centrifugation, they were dispersed in Triton-buffer solution. After extensive high-

speed centrifugation of the solution, gonadotrophin-binding activity was found only in the supernatant solution. Some loss of binding capacity occurred on solubilization, as the final solution had only about one third the binding capacity of the original particles and this was also associated with decrease in the association constant. Thus although all of the receptor may have been solubilized there well may have been a conformational change in the protein. The soluble receptors, however, still appeared to retain their hormonal specificity. Isolation of the receptors by this type of procedure should not only be a great help in their further characterization and investigation of the way in which they function but also enhance their usefulness in the development of methods for assay of gonadotrophins.

A second report, by Milin and Roy, is concerned with androgen-stimulated synthesis of globulin in rat liver. Hepatic tissue contains a "receptor" which on ultracentrifugation is different from the androgen-binding protein present in blood. Earlier studies showed the absence of this "receptor" in liver tissue of female and immature male rats and Milin and Roy used for their new studies a strain of rats which show no response to androgen because of a genetic defect. None of the "receptor" was detected by ultracentrifugation of the liver from these animals nor did they produce significant amounts of the globulin.

Tees. As they develop they drift southwards as larvae and onshore into the north Yorkshire nursery ground. Here, after metamorphosis, the 0 group fish establish a depth distribution related to size. The 0 group over-winter in the nursery ground and begin the migration to the parent spawning ground as 1 group fish.

In her talk on local and annual variations in the recruitment of the limpets *Patella vulgata* and *P. aspera*, Dr R. S. Bowman (University of Leeds) described how, at Robin Hood's Bay, Yorkshire, she chose fixed-site monitoring as being a more sensitive and accurate method of measuring input of settlers than random sampling. Annual input varies widely in both species; this, she said, seems to result from the effect of summer air temperatures on gonadal development in *P. aspera* and on the susceptibility of newly-settled spat to hard frost in *P. vulgata*.

Dr J. B. Buchanan (University of Newcastle upon Tyne) discussed the life history of the burrowing decapod crustacean *Calocaris macandreae*. A monitoring programme off the Northumberland coast over a ten-year period has shown that this species holds its population density at an average of eighteen individuals per square metre, with a coefficient of variation of only 5.4 per cent. An analysis of grab samples revealed that the species is evenly distributed on the ground and experiments in the aquarium tanks of the Dove Marine Laboratory showed it to defend individual territories which are in the form of burrow systems.

Mr R. Foster-Smith (University of Newcastle upon Tyne) described the effects of turbidity on the feeding of *Mytilus edulis*, *Cerastoderma edule* and *Venerupis pullastra*. He has found that *Mytilus* is more capable than the other species of withstanding high concentrations of suspended matter, and does this by rejecting a greater proportion of filtered material than either *Cerastoderma* or *Venerupis*.

The special lecture, given by Dr J. A. Allen (University of Newcastle upon Tyne), was also devoted to benthic molluscs but to a group far less familiar than Mr Foster-Smith's cockles and mussels, namely the bivalves of the abyss. Here the bivalve fauna is dominated by the deposit feeding sub-class Protobranchia, whose food consists largely of refractile scleroproteins such as are found in the matrix of the exoskeletons of dead diatoms or Foraminifera. Morphological adaptations invariably include the elongation of the hind gut, thus increasing digestion time. Growth rate of these animals is very low but the life span may be commensurately long, sometimes in excess of a hundred years, with maturity attained at the end of the life span.

PHYSICS EXHIBITION

1+1=2+?

For the first time, the annual Physics Exhibition, organized by the British Institute of Physics, was held last week at Earl's Court, London, rather than at the more inaccessible, and inelegant, Alexandra Palace. Also, on this occasion the exhibition took place at the same time and in the same place (though on a different floor) as Labex International, an exhibition of scientific instruments.

In the past few years the Physics Exhibition has experienced a disappointing decrease in the number of exhibitors, and at least one of the motives for linking up with Labex International was to try to rectify this state of affairs. Dr L. Cohen, secretary of the Institute of Physics, said last week that the number of exhibitors was about the same as last year, possibly slightly greater. Nevertheless the recent decision to hold the exhibition only every two years from now on still stands.

Several of the exhibits at the Physics Exhibition are traditionally educational in character, and this year one of the most interesting was an inexpensive apparatus for measuring the speed of light. It was devised by the Physics Department of King's College, London, and can be built for about £25 together with the apparatus usually found in school science laboratories (see *J. Phys. E.*, 5, 1142; 1972). The principle is to modulate the output from a gallium arsenide-phosphide light-emitting diode and to compare the phase of this output with the phase of the signal detected at another diode a metre or two away.

Another inexpensive piece of educa-

tional equipment on show was the sound level detector developed for the Technology Foundation Course of the Open University. This is being used by students to measure vehicle and machinery noise as part of their practical work.

One of the more extensive displays was undoubtedly that put on by Harwell to illustrate the many facets of research in which that establishment is now engaged. Among the activities publicized were research on multifilamentary Nb₃Sn superconductors and the work of the establishment's non-destructive testing centre.

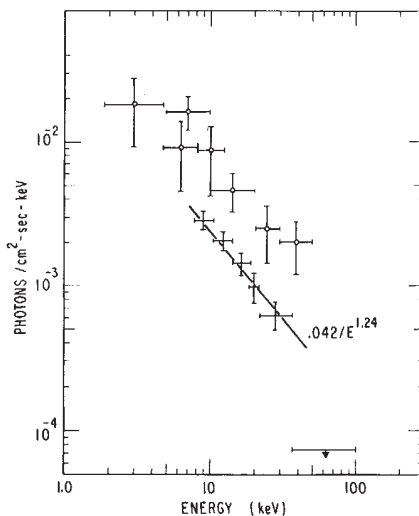
Although the a.c. linear motor is now quite celebrated for its role in high speed transport, d.c. linear motors are less well known even though they have important advantages in the context of, for example, machine tools. In such a motor built at the Wolfson Centre for the Technology of Soft Magnetic Materials by the University of Wales Institute of Science and Technology and shown at the exhibition, the field excitation is provided by ferrite permanent magnets and the armature is a steel bar wound with copper wire. The speed of the motor can be varied simply by altering the current flowing through the armature.

Time alone will tell to what extent the next Physics Exhibition in 1975 will benefit from the decision to hold the event less frequently. It is certainly true to say, however, that many people who have not visited the exhibition every year in the past will be more encouraged to do so regularly in the future, and this should have a beneficial effect on the number of exhibitors.

SMC X-1 Observed at Energies > 7 keV

OBSERVATIONS of the binary X-ray source SMC X-1 are reported in next Monday's *Nature Physical Science* (April 23). These observations, made from the satellite OSO-7, extend to energies greater than 7 keV, thus complementing the Uhuru observations in the range 2 to 6 keV. The period found is consistent with the 3.8927 day period of the Uhuru data.

Ulmer *et al.* have also extended the known spectrum of SMC X-1, by means of the OSO-7 observations, to 35 keV (see diagram). The best fit to their data is a slope of $0.04E^{-1.24 \pm 0.09}$ photon $\text{cm}^{-2} \text{s}^{-1} \text{keV}^{-1}$, which agrees well with the rocket data of Price *et al.* (*Astrophys. J. Letters*, 168, L7; 1971) which were obtained in 1970. The integrated luminosity from 7 to 35 keV is $4.5 \times 10^{38} \text{ erg s}^{-1}$, assuming a distance of 63 Mpc, and there is evidence, say Ulmer *et al.*, that the spectrum steepens between 35 and 60 keV.



Spectrum of SMC X-1. +, OSO-7 observations reported in next Monday's *Nature Physical Science*; o, rocket observations made in September 1970 (see text).

CHEMICAL SOCIETY

Pollution and Resources Discussed at Swansea

from Correspondents

BETWEEN March 26 and 30 the Chemical Society held its annual congress in Swansea. Two of the symposia were particularly topical, for they dealt with environmental pollution analysis and the winning of metals from lower grade sources.

Atmospheric Pollution

At the well attended meeting of the Analytical Division, participants listened to and discussed ten contributions which were chiefly concerned with atmospheric pollution. The scene for the details of scientific procedure and instrumentation was set by a particularly fine keynote paper by Dr L. A. Clarenburg (Rotterdam Public Health Department). Hinting that analytical methods were already fairly satisfactory for most pollutants and that the search for new ones had a low priority, he stressed the real environmental problem of building them into an integrated regional industrial system for what he termed "real-time" analyses to study the progressive build-up of potential pollution incidents.

Quoting the example of the Dutch experiments at Rijnmond and Vlaardingen, Dr Clarenburg showed how problems of odour in the former and SO_2 in the latter had been studied against the natural environmental parameters of time of year and day, temperature, humidity, wind speed, light intensity, natural particulate levels and so on. In both instances continuous monitoring signals from strategically sited automatic sensors were fed into a central computer. This machine was programmed to assess the data against the natural parameters and produce an early warning of potential incidents in sufficient time to allow the industrial concerns in the area to moderate or, if necessary, shut down their appropriate activities, thus minimizing the buildup of the symptoms and preventing further progression of the potential incident. Some particularly interesting points were raised, for example, the synergistic effects of multiple odours and environmental parameters, the supersensitivity of the human nose and the psychological as opposed to the physiological aspects of smell. (His recent report in *Zeitschrift für Analytische Chemie*, **263**, 198; 1973 merits reading).

In a fascinating contribution on pollution studies of the upper atmosphere, using Concorde 002 as the flying "laboratory bench" and submillimetre interferometric spectrometry as the technique, Dr N. W. B. Stone (National Physical Laboratory, Teddington) discussed the potential "pollution" of the stratosphere by water vapour from

trans-sonic jet aircraft. What effect would the injection of considerable amounts of water vapour into the lower and intermediate layers of the stratosphere have? What are the present levels? Had they been seriously influenced already by military supersonic aircraft? Would the injection of hydrocarbons, nitrogen oxides and so on from jet engines seriously upset the photoionization balance? A sensitive technique seems to be available here for the study of the natural levels before trans-sonic flights can directly disturb the situation.

Multiple analyses of atmospheric levels of ozone, hydrocarbons, sulphur dioxide, carbon monoxide, nitrogen oxides and so on by gas liquid chromatography using flame ionization and chemiluminescence detector systems in an automated apparatus were described by Dr R. G. Derwent (Warren Spring Laboratory, Stevenage). In the study of traffic pollutants he finds that procedures made selective by adsorption stages inevitably lead to loss of detailed information, whereas those based on properties, such as chemiluminescence of gas phase reactions, are usually selective, sensitive and fast responding; for example,



Much interest was evoked by Dr Derwent's description of the 0.132 p.p.m. of ozone found in the atmosphere of London by this technique. This is slightly above the upper "safe" limit of 0.1 p.p.m. The lachrymatory effect of ozone is felt at 0.2 p.p.m. and the "safe" concentration of 0.1 p.p.m. is based on an exposure for 1 h. Little is known of the effects of longer exposure to lower concentrations, but it might be significant that in Los Angeles, where more abundant sunshine could lead to longer exposure to ozone, the limit has been set at 0.08 p.p.m.

The three contributions mentioned here were typical of the other seven. Less attention was, however, paid to water pollution, but the keynote paper from Dr A. C. Docherty (ICI Agricultural Division, Billingham) more than redressed this in its quality.

Chemistry and Metallurgy

When the late Sir Ronald Nyholm proposed the symposium on metals from lower grade sources he felt that it would bring together chemists and metallurgists to discuss problems to which each could contribute. Furthermore, it would demonstrate the interdisciplinary nature of much of modern science and techno-

logy, particularly in its applied aspects. The result provided good fare for substantial audiences by no means confined to those professionally involved.

Apart from the question of funding the massive, high-risk cost of modern large-scale mining and metallurgical operations, the chief problems facing the industry are similar to those of the chemical process industry, namely, exploration and depletion of reserves; the environmental impact of large-scale mining and metallurgical processing, including the problem of the emission of SO_2 ; and the accurate technico-economic appreciation of new process technology as compared with existing technology. In the production of non-ferrous metals, environmental pressures have greatly accelerated the rate of commercialization of the newer pyrometallurgical processes, and interesting controversy continues on the possible future scope of hydrometallurgical techniques combined with solvent extraction and/or electrolysis. A little further into the future, the possible impact of chlorination technology arouses keen discussion.

Growth and Reserves

Metal usage is an essential feature of industrial society and Dr A. J. Robinson (Warren Spring Laboratory) pointed out that consumption of most metals doubles every 16 to 20 years, with aluminium and copper much above the average. The resultant large scale of mining and metallurgical operations has been one of the principal factors in maintaining real prices below those of 60 years ago. Drs B. G. Baldwin and D. Christie (British Steel Corporation) pointed out that an iron blast furnace producing 1,000 tons a day would have been considered normal in the 1930s, but daily outputs of 7,500 tons are not uncommon in Japan today and predictions for the 1980s run as high as 16,000 tons a day. Their contribution emphasized the value of accurate models of iron blast furnace performance to the complex problems, among others, of furnace burden preparation and the ore purchasing policy of the British Steel Corporation. Unlike some non-ferrous ores, plentiful supplies of rich iron ore remain available but they are distant and present logistic problems, and their scale of usage and relatively low cost leaves little margin for technical error.

Copper ores are trending sharply downwards in grade, but Mr M. J. Cahalan (Rio Tinto-Zinc) pointed out that proven reserves at current prices have kept pace with demand; he also noted that the high cost of exploration deters the extension of proven reserves beyond 50 years. In working leaner ores, Dr Robinson indicated some of the advances that have been necessary in selective flotation since it was first commercialized in Australia in 1909, in

milling and materials handling, and in the highly sophisticated methods of analytical control now in use.

Although flotation research continues to be active, Dr Robinson thinks that specific flocculants to deal with particles of 1–20 μm might become important. These techniques, and improved large-scale mining methods, are making possible the economic working of lower grade ore bodies and Dr D. Slater (Institute of Geological Sciences) reviewed mining possibilities in Britain from the geological standpoint, mentioning some of the newer analytical and theoretical techniques which are being brought to bear on the problem. Copper, tin and possibly nickel seemed to be the likeliest possibilities, but it seems unlikely that the recently developed Irish lead–zinc deposits extend across the Irish Sea. Dr T. W. Farthing (Wolverhampton Metal Holdings) gave a fascinating account of the economics and technology of the secondary and scrap recovery industries, but he pointed out that the highly fragmented structure of the collection part of the industry militates against the kind of technological improvement recently made on the more integrated refining side.

Process Technology

Process technology has evolved rapidly during the past 20 years and Professors F. D. Richardson (Imperial College) and P. A. Young (University of Leeds) presented some aspects of their physio-chemical investigations typical of those necessary as a foundation for advance. Professor Young and others showed the recurrent importance of accurate phase diagrams for the interpretation of the behaviour of slag systems. Over the whole range of metals, pyrometallurgical smelting continues to predominate with the newer processes offering solutions to the problem of SO_2 emission, which is inseparable from the smelting of sulphide concentrates. Dr Robinson and Mr Cahalan reviewed newer copper processes, including the Outokumpu flash smelting process, the Kaldor rotary converter and continuous processes of the Worera, Mitsubishi and Noranda types, and analysed conditions for disposal of sulphur as sulphuric acid or, potentially, as elemental sulphur. Mr C. F. Harris reviewed the Imperial Smelting lead–zinc blast furnace, emphasizing particularly its complementary rôle to Waelz treatment and slag fuming in the treatment of low grade and complex materials, and the environmentally acceptable nature of the slag produced by the furnace. Recently escalating coke prices are stimulating processes based on electrical energy for zinc and copper. Mrs Baldwin and Christie showed that this is not a dominant factor in iron production, but

the account by Drs J. B. Cartwright and G. B. M. Eastmond of electrosag refining illustrated how development is making this process applicable to lower alloy steels.

The combination of hydrometallurgical leaching with solvent extraction is attractive environmentally and Mr A. W. Fletcher (Warren Spring Laboratory) related how considerable research and development in this field is beginning to have an impact on copper processing, topics also discussed by Dr Robinson and Mr Cahalan. Mr Fletcher reviewed the “intense” Forward, Arbiter and Cymet (FeCl_3) processes, all of which utilize a leaching stage under rigorous conditions followed by solvent extraction. He also reviewed the future possibilities of biological leaching as a means of economically working low grade ores and dumps. Work on all of these processes is stimulated by the in-

creasingly stringent emission and effluent controls being placed on conventional smelters.

Chlorination offers many theoretical advantages for metal extraction and some of these were exemplified in the lucid review by Dr S. C. Townshend (International Nickel) of very recent laboratory studies on the hitherto intractable limonite nickel materials. A more general review of chloride processes was given by Mr D. V. Jackson (Warren Spring Laboratory), who pointed out that wider adoption of such technology had been hampered by construction problems with materials; adequate pilot-scale demonstration plants were particularly necessary to overcome fears and objections on this score. The outcome of Alcoa's new route to aluminium, based on AlCl_3 , would be awaited with interest from this viewpoint.

Gravity Surveys in Scotland

ALTHOUGH the geology of the Tertiary intrusive complexes of Skye, Mull and Ardnamurchan (North-west Scotland) has received considerable attention for many decades, these bodies have, perhaps surprisingly, been subject to relatively little geophysical investigation. In next Monday's *Nature Physical Science* (April 23), however, this situation is remedied to some extent by Bott and Tuson who report the results and interpretations of gravity surveys carried out over all three complexes.

In all cases the Bouguer anomalies are positive, being particularly large over Skye and Mull, and thus indicate the presence of large underlying volumes of relatively dense igneous material. In the Skye intrusive complex almost equal surface areas are occupied by basic-ultrabasic and granitic plutonic rocks; but the Bouguer anomaly indicates a very different picture at depth. After subtraction of the estimated regional background, the maximum amplitude of the near-circular anomaly is +53 mGal. Because basic and ultrabasic igneous rocks are usually denser than the metamorphic basement, whereas granitic rocks are less dense, Bott and Tuson infer that at depth basic-ultrabasic igneous rocks predominate.

The principal density contrast giving rise to the positive anomaly over Skye must therefore be that between the basic-ultrabasic rocks and the gneissose basement. Looking at the situation in terms of a detailed model, Bott and Tuson conclude that the minimum possible contrast between complex and basement must be 0.18 g cm^{-3} . Although the precise density of the basic-ultrabasic body is not known, this implies that the overall density of the intrusive complex is greater than that (2.95 g cm^{-3}) of the average gabbro represented by surface

samples. Bott and Tuson thus assign a minimum density of 3.01 g cm^{-3} to the subsurface body which is taken to have a roughly rectangular plan and to widen gradually from the surface to the base at a depth of about 14 km.

By this interpretation the subsurface intrusive body has a volume of about 3,500 km^3 of which less than 5 per cent can be granite. Because no interpretation of gravity is unique, Bott and Tuson also offer a second model in which the non-granitic part of the intrusion has a density of 3.15 g cm^{-3} , the maximum depth is 5.5 km, and the widening with depth is more rapid than in the first model. In this case, the intrusive volume is about 1,500 km^3 of which granite forms about 7 per cent. Between these two extremes there is a whole range of models consistent with the Bouguer anomaly, but in no case can the volumetric proportion of the granite in the intrusion exceed about 8 per cent.

The surface geology of the Mull complex is quite different from that of Skye, granitic rocks being most abundant. Nevertheless, the Bouguer anomaly over Mull is similar in shape, area and amplitude to that over Skye, indicating a similar subsurface structure. Again, therefore, non-granitic rocks must predominate in the intrusive body. (In the case of Ardnamurchan the gravity is not well enough defined for a full interpretation to be possible.) As Bott and Tuson point out, the unexpectedly large volumes of basic-ultrabasic material beneath Skye and Mull make it more difficult to explain the origin of the intrusive complexes. Their own suggestion (which agrees with a previous one based on the geology alone) is that magma has risen from the lower crust by repeated stoping and/or cauldron subsidence.

Environmental Archaeology in the Western Negev

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The extent of change in environment and settlement during the past 1,500 years was demonstrated last year in the semi-arid zone of the western Negev by a multi-disciplinary British team.

In recent years, European archaeology has witnessed the development of new methods of research, adding to the study of man's artefactual evidence a study of ancient environment, its ecology, climate, geomorphology and allied subjects. In Western Asia, long the home of great archaeological discoveries, this newer study has, however, been slow to develop. Frequently the scientist has been called on only as an adjunct to the older study, and his work published in appendices as a sop to the scientific world. The greater discipline now demanded within the subject calls for a more consolidated examination of areas in Western Asia so that the interpretation of the archaeological evidence may be placed on a more rational footing. The prime movers in this field of study in the Near East have been particularly Vita Finzi and Higgs¹ as well as Legge *et al.*².

In a further attempt to redress this balance, a survey was begun in Israel during the summer of 1972, the intention being to create a team of scientists whose interests also touched on the archaeology of the region; thus the team would comprise a botanist, chemist, geomorphologist and environmental specialist together with the archaeologist. The ancient environment would then be studied as a prerequisite to the study of man³. Such an interdisciplinary team would examine an area of marginal agriculture, where the environmental factors would be most critical, in order to set parameters on its modern and ancient environment, to study the present flora and to compare this with residual ancient pollens and charcoal from datable deposits, to follow the geomorphological history of the area in its construction and appearance in ancient times, to analyse its soils for chemical composition, particle size and history, and to focus these disciplines on archaeological sites in the area.

The area selected was the region around Tell Fara, in the Negev desert near Gaza. An isolated semi-arid region bisected by deep dry river beds, the Negev lies in the southern coastal plain of Israel, south of Tel Aviv and south-east of Gaza. There were two reasons for this choice. First, this is an area where normal agricultural methods produce an extremely precarious economy, balanced between scant success and abysmal failure. Thus minor fluctuations can have far-reaching consequences in settlement patterns and land use. Second, it has been known in the past as an area rich in archaeological material, dominated as it is by a typical Bronze Age/Iron Age site⁴ (Tell Fara) from 1800 BC to the Roman period. In the surrounding areas, pre-urban settlements have been discovered which date from the fourth millenium BC. Previous excavators

have also believed that even earlier material from the Palaeolithic period (stretching back many millenia before 12,000 BC) is present. A correlation of these deposits, if located, might well offer suitable dating criteria for the environmental research.

Geology

The results are preliminary, but already vindicate the theory. Geologically, the lowest rocks in the area are a series of fossil dunes forming a series of headlands on which the tell itself stands. In part these dunes are overlaid by a bed of heavy gravels, some of the larger cobbles of which show signs of marine battering. Both of these strata are partially covered by a very heavy deposit of loess, the uppermost layers of which now form the country surface of the western Negev. Erosionally the area is dominated by the Wadi Ghuzzeh (Nahal Basor), a situation which can be proved to have existed for at least the past 50,000 yr as it is seen to be bounded by ancient sites. At present the level of the wadi is on the gravel, well below the loess strata.

In winter, the wadi is fed from several deep gullies extending back into the relatively soft loess and dissecting the country surface thus creating an extensive area of "badlands". The morphology of this area is extremely complex, with much redeposition of material. In general, however, there is a history of an underlying system of erosion and deposition of the loess during the past 50,000 yr which has produced a series of roughly horizontal layers to a total depth of some 30 m. Several of



Fig. 1 Area around Tell Fara, Israel. In the right middle ground are the soundings of the Levallois/Mousterian floor, beneath some 30 m of loess.



Fig. 2 Tell Fara (left foreground). Left distance, fossil dunes; centre Wadi Ghuzzeh; right distance, loess badlands. Note the large stands of young tamarisk in the wadi.

these strata are heavily oxidized and weathered bands, indicating periods of arrested deposition, but superficially at least these bands are very similar in texture and appearance to the seasonally arid, weathered, tropical soils. (For a discussion of palaeosols on this area see Dan and Yallon⁵.) A stratigraphic column of these deposits was traced and samples collected which should provide at least a relative sequence of soil data for the evaluation of the changing face of the Negev.

Botany

As no exhaustive collection of plant material is available in Britain, as complete a collection as possible was made of the present summer flora, which amounted to the striking number of 107 species. In spite of the obvious lacuna in spring annuals, the figure was higher than expected. These were pressed in the field, their fruits were pickled and their seeds collected. Secondary wood from trees in the wood was sampled. The classification of the plants is now complete and thus forms both a reference collection for comparison with ancient plant evidence and a control on the modern environment.

The plants appeared in very marked zones, some of which ran parallel to the water course and some at right angles to it. The obvious explanation of the former is water availability, but for the latter a different solution must be found, possibly a combination of the mechanical and chemical composition of the soil.

Two trees were noticed, *Acacia raddiana* and *Tamarix nilotica*. These two have frequently been recorded in this part of the Negev⁶. In the area surveyed, however, only one mature specimen of acacia was found, and only two or three old tamarisk. On the other hand, thousands of saplings of acacia testified its natural position in the area, and large stands of young tamarisk dominated the wadi floor. Clearly this new growth indicates some fairly recent change in the area.

Archaeology

Several sites were located and investigated, two of which provided the necessary termini for the geomorphological column. Near the base of the loess were several sites dating from the Middle Palaeolithic (Levallois/Mousterian) period, at least one of which was an undisturbed deposit with bone

and charcoal appearing in equal quantity with the flint tools. From these sites a picture can be built up of the pre-agricultural Negev, its surface and appearance. Doubtless these sites are concentrated on an ancient water hole, for copious faunal remains of primitive cattle (*Bos*) and large red deer (*Cervus* (?) *elaphus*) occurring in the deposit show it to be an open butchering site. Analysis of the charcoal *in situ* and of the pollen now found to be present in the stratigraphic column will render an approximation of the contemporary flora. This site then and its data give a lower terminus to the survey of about 50,000 BP.

At the other extreme, on the top of the present country surface were the remains of a Byzantine farmstead, once a prosperous settlement, its buildings, cisterns and fields now almost completely eroded away. Pottery from the site dates it as the period of Justinian, around 550 AD. The walls of the buildings had been undermined by erosion and now lay collapsed in the resulting gullies. The silt dams constructed across the ravines below to collect the eroded soil each year and thus snatching a harvest from the low rainfall have long since been breached. Only a once-subterranean cistern, now standing above ground level, remains intact. Clearly, although the pre-Byzantine period may have been one of deposition, the post-Byzantine period was one of fierce erosion. It will be in the period between 50,000 yr and 2,000 yr ago that environmental evidence will have the most significant archaeological application. The necessary laboratory work involves the extraction and determination of pollen from the samples covering this period. Difficulties arose in the extraction of pollen from loess deposits, but these have now been overcome by the application of flotation techniques⁷ and ancient pollen is being recovered. The programme of soil analysis must also be completed before any interpretation is attempted. On the other hand, the past 1,500 yr may be used as a model for the type of results that one might hope to obtain. During this past one and a half millennia three environmental periods can be distinguished in the area.

Three Periods

The remains of the Byzantine farmstead show that in its day it covered an extensive area of some 5,000 m² of buildings. Its farmed land probably extended beyond the use of silt dams

to the cultivation of the country surface. This was the last phase of the heyday of Negev agriculture. Either through economic pressure, or more probably political necessity, this style of agriculture died out following the Omayyid invasion of the mid-seventh century, never to be repeated. It was at this time that the Bedouin, no doubt always present in the fringes of these previously semi-urban areas, were drawn into the vacuum. These Bedouin are not the camel nomads of the East, but semi-nomadic roving pastoralists, living from their herds of sheep and goats, and from scant farming. The natural result of this type of exploitation was overgrazing and denudation of trees with consequent floral deterioration and soil erosion⁸. Evidence of this new erosional phase is to be seen in the almost total annihilation of the Byzantine buildings by a dendritic system of gullies which has eaten back into the country surface during this time.

The arid position thus promoted has persisted in the area until very recently. Photographs taken of the area during the 1920s and 1930s show a treeless, parched landscape⁴; but now the area has changed to a ground cover which even in summer can be lush. Because the Israeli government has embarked on a resettlement plan for the Bedouin during the past few years, they have been discouraged from using the area. As a result, the new natural growth is dramatic, hence the large stands of immature acacia and young tamarisk that have

recently begun to dominate the area once more. Although the flora can never return accurately to its pre-occupational ecology, there is a tendency for natural flora to recur at this time.

This model has illustrated the extent of change in environment and settlement over the past 1,500 yr, which would itself result in archaeologically significant alterations. To quantify these interdependent factors for earlier periods will require more research in the area, and to this end a further season is planned for 1973.

The expedition is grateful to Educational Expeditions International, Belmont, Mass., for their generous support for the 1972 season.

¹ Vita Finzi, C., and Higgs, E. S., *Proc. Prehist. Soc.*, 1 (1970).

² Legge, A. J., et al., in *Papers in Economic Prehistory* (edit. by Higgs, E. S.) (Cambridge, 1972).

³ Butzer, K. W., *Environment and Archaeology* (Methuen, 1972).

⁴ Petrie, F., *Beth Pelet I* (Quaritch, London, 1930).

⁵ Dan, J., and Yallon, D. H., in *Paleopedology* (edit. by Yallon, D. H.) (Israel University Press, 1971).

⁶ Zohary, M., *Plant Life of Palestine, Israel and Jordan*, 143 (Ronald Press, New York, 1962).

⁷ Hubbard, R., *Proc. Roy. Anthropol. Inst. Great Britain and Ireland*, 79 (1971).

⁸ Evenari, M., Shanan, L., and Tadmor, N., *The Negev, The Challenge of a Desert*, 326 (Oxford University Press, 1971).

Algeria's Answer to a Manpower Problem

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Algeria is combating a severe shortage of skilled people by means of technological institutes geared closely to the nation's manpower requirements.

ALGERIA'S biggest problem on gaining independence in 1962 was the severe shortage of skilled manpower caused by the European exodus. High priority was given to the expansion of education to create a class capable of doing both the jobs previously done by Europeans and also the new administrative and technical jobs which the demand for development created. Between 1963 and 1969 the number of children in primary education doubled—the percentage of children in primary schools was raised from 25% in 1962 to 53.71% in 1970—and the most realistic forecasts aim at achieving universal primary education in the early 1980s. Expansion in secondary education has been even more rapid: between 1963 and 1969 the number attending quadrupled. The overall figures for university expansion too are impressive: between 1963 and 1969 the numbers increased ten-fold.

This massive expansion could only have been carried out with massive investment. In 1970 Algeria devoted 23% of the national budget to education and under the Four Year Plan (1970–1973) 11% of the total investment is for education and training.

But these overall figures conceal an alarming wastage rate. Only 54% of the children entering primary schools

complete their primary studies, and, of these, about 20% go to secondary schools; only 15% of children entering secondary schools complete the 7 years. Wastage at university level is also high; only 10% complete their course. The authorities are obviously concerned by this wastage and the great investment that shows so little return. In some fields the situation has even worsened. Rather than encouraging their children to become technicians or skilled workers, many parents prefer to send them on a fruitless chase after more "respectable" paper qualifications, leading frequently to unemployment. The educational system does not produce the kind of manpower which the economy needs.

The new needs engendered by the Four Year Plan and the replacement of foreign personnel by nationals are enormous. Algeria has engaged in an ambitious plan of development whose success depends on a considerable increase in the number of technicians, scientists and administrators, and yet is unable at the moment to meet even the demands of the present. The demand for advanced training in engineering (21,000) is three times greater than existing educational facilities could satisfy (7,000). Whatever progress education has achieved it is completely out of tune with economic reality. The real problems of development cannot be solved by the mindless expansion of a system that had been designed to meet the needs of a French colony. An initial phase of educational expansion should give way to an educational system tailored to the economy, culture and social needs of Algeria. A National Commission for Educational Reform was set up, but its proposals will not be implemented before the end of the period

of the Four Year Plan. Trained manpower requirements, however, must be met within this period. Drastic change in institutions, methods, contents and priorities was therefore vital and a short cut had to be taken.

Setting up Institutes

Alongside the existing educational system a new training system has been set up to provide, within the shortest possible time, those specialists most urgently needed for Algeria's development.

In 1970, the Institutes of Technology were opened in response to these needs. They are to offer training on a large scale based both quantitatively (through the calculation of numbers required at different levels of skills) and qualitatively (through the adaptation of content and methods of specific requirements) on manpower needs. The institutes are thus to train 17,710 people at an intermediate level and 9,930 at a higher level for the industrial sector, and 55,200 skilled workers and people at an intermediate level and 15,910 people at a higher level for the social sector, within the period of the Four Year Plan.

Such a system will only be temporary; the reformed school system should eventually take over its functions and the institutes will then become in-service training centres or will provide refresher courses for those already employed.

Accordingly, no investment was made in building and equipment; instead existing facilities like vacant premises, buildings not utilized to their fullest extent, and disused army barracks were called into play as much as possible. Only DA 200 million (£17 million) was allocated to the institutes for capital expenditure whereas DA 2,718 million (£230 million) went to the national education sector.

As the training provided must be adjusted to manpower demand, job analysis studies are used to define the tasks; from these can be obtained details of responsibilities, skills and personal qualities required for a particular occupation. These observations are then translated into training objectives and syllabuses. The learning process follows the principles of on-the-job training; practical work and an outside assignment form an integral part of the course and field-work underlies theory.

Recruitment is not based on a diploma, but on the applicant's general educational level, and on his motivation,

aptitudes and desire to work; students are mostly secondary school drop-outs who, until now, have found it almost impossible to find themselves a niche.

The project was not seriously examined until 1969 when a commission was set up; this included representatives of all the ministries involved, of administrative and business concerns and of educational organizations. Eight project chiefs to be responsible for all the preliminary work involved in the actual launching of these institutes were appointed. Seventeen categories of institute were provided for: the Institutes of Agriculture (engineers; one), Agriculture (technicians; seven), Chemistry (one), Commerce (one), National Education (twenty-one), Electromechanical Maintenance (one), Finance and Accounting (one), Hotel and Tourism Trade (one), Hotel Trade (one), Agricultural and Food Industries (one), Metallurgy and Mining (five), Planning Techniques (one), Public Health (three), Telecommunications and Electronics (one), Public Buildings and Works (one), Topography (one) and Youth Services (under study).

Administering the Institutes

The first institute launched was the Mostaganem Technological Institute of Agriculture; it was opened in January 1970. It provides training for higher level engineers and specialists for the agricultural sector. Since then thirty-three others have been set up, including twenty-one Ecoles Normales which have been converted into Teacher Training Institutes.

The managing body of each institute is the Administrative Council; one of its chief functions is to consider job placement of graduate students. Its members include, among others, representatives of the prospective employers: the "users"—the involvement of the users in the planning and running of these institutes is one of their distinctive features. This makes sure that the training provided meets the needs and will also ensure a balance of supply and demand on the labour market.

All the directors of these institutes are Algerian and higher education graduates. The teachers are either teachers from traditional schools, specialists from enterprises who work part-time in the institutes, or foreigners recruited under technical assistance agreements. The



Fig. 1 The Ben Aknoun complex in Algiers. This houses three institutes—the Technological Institute of Commerce, the Technological Institute of Finance and Accounting and the Institute of Planning Techniques.

students work in small groups and the staff-student ratio is one to ten. Approximately 1,500 teachers are employed at present.

As the student is trained for a specific job he is more or less considered as an employee: he receives a training allowance and benefits from the same social security scheme as salaried personnel in public service. Furthermore, contracts are entered into by the institutes or the student, on the one hand, and the enterprise or public service on the other, to guarantee employment to the student trainees on completion of training. The graduate trainees are, however, required to work in the service of the enterprise to which they have been appointed for a period corresponding to the length of training.

Achievements

Looking at what has been achieved after a year or so of existence, it is clear that, in spite of all the difficulties encountered, and the setbacks and delays, the implementation of the programme is well advanced and a considerable amount of experience has been gained.

Of the candidates who applied following a publicity campaign in the summer of 1969, 18,305 were selected on the basis that they had at least reached the standard of a fourth form in a secondary school, that they were not employed and were not enrolled in a school. Their general and specific attitudes were tested; then during the summer of 1970 a two month course was organized. After this course tests were given and their results used for selection of the students to be admitted to the institutes the following September.

There are at present more than 13,000 trainees on courses at these institutes, including 7,692 technicians, 4,385 engineers and cadres and 1,182 qualified workers. Of the applicants who attended the preliminary course during the summer of 1970, it seems that 92% of the boys were under 22 (69% under 20), whereas 96% of the girls (who represent only 9% of the whole group) were under 22 (79% being under 20). The proportion of younger students is likely to increase as the scheme is more fully implemented. The launching of several institutes has had to be postponed because of administrative difficulties or delays suffered in the construction or the conversion of buildings. These delays are mostly a consequence of the shortage in qualified personnel in the ministries to carry out the necessary manpower surveys, translate this information into training objectives, determine the length of training, decide on the appropriate methods of teaching and on the syllabus, and so on.

Successes and Difficulties

If it is possible, after nearly two years of existence, to take stock of the numbers of institutes opened and the numbers enrolled, it is too early yet to assess the achievements qualitatively. As the purpose of these institutes is to train personnel who will be directly adjusted to a particular job, it will not be possible to assess the overall value of the training provided until these trainees are engaged in production. A number of positive aspects can already be stressed nonetheless: periods of integrated practical training have been organized and have been welcomed by the public services or concerns, study trips have been conducted throughout Algeria; and formal lectures have been replaced by work in small groups. Most of the trainees readily accepted the methods of active education. A number of difficulties have, however, been encountered as regards self-assessment and self-discipline, and there is less satisfaction with the results of those converted institutions where the project is run on more traditional lines.

The principal difficulties have arisen from the "diploma"

issue; also the rules governing the students within the Civil Service, after they have completed their training, have not turned out to be easy to define.

Great difficulties have also been encountered as regards credits and the recruitment of staff. It was impossible to find locally a sufficient number of qualified teachers and it was therefore necessary to rely heavily on foreign assistance. In the long run the most important problem will be the training of local instructors to replace the non-Algerians. Several institutes have made provisions to draw instructors from among their old students, and we would also like priority to be given to the institutes when university students are appointed to the public services for their two years of national service, during which they are obliged to do a job assigned to them within their speciality.

There have been criticisms of costs and charges of excessive specialization. Costs are certainly very high, but it has not yet been possible to carry out an analysis of the cost per student trained. One must, however, bear in mind that these students will be operational as soon as they have completed their training, for the training has been based on a systematic analysis of the job concerned in close cooperation with the users: this should reduce wastage and therefore diminish the cost.

The other criticism relates to excessive specialization. Narrow specialization is a necessity if Algeria really wants to fill the shortage in qualified manpower and wants its industry to take off. A more general academic form of education would place too much emphasis on the individual as such and not enough on the productive worker; there is need for technical education related to the performance of a job: this is the first priority and it is only an exceptional response to exceptional circumstances. With the eventual return to a normal situation, the institutes will no longer have to maintain such a fast rhythm and will provide further training and retraining of those already employed. The vocational training which they offer will also be supplemented by programmes of general educational value produced by the National Centre for Generalized Education through radio, television, the press and correspondence courses.

The Institutes of Technology are an educational experiment which cannot be developed in a void; they must be firmly rooted in the life directly around them. They must contribute to improving the standard of work and life in their areas, and to the building up of a socialist society. In fact, they are already beginning to do this and the Mostaganem Institute of Agriculture, for instance, has already organized numerous periods of field work in agricultural estates under workers' management. Also the fact that the graduate trainees are bound under a contract to work in the service of the enterprise to which they have been appointed for a given period will help to shift the focus of development away from the urban areas to the rural or poorer areas.

The Algerian experiment might well be of interest to other countries in the Third World where the same problems need to be tackled: problems like school drop-outs or even school-leavers holding diplomas and flocking to the cities but unable to find employment, most of them being functionally useless because they come from a non-functional educational background. At the same time, there is an acute shortage of the skilled manpower, especially of intermediate level, required to supply the needs of the economy and to break out of underdevelopment.

Our ambition is thus not only to train sufficient numbers of personnel of sufficient quality to meet the needs of the economy, to innovate in teaching methods and programme contents and give further opportunity to those denied it, but also, if we achieve these aims, to serve as a creative model for other developing countries which have the same problems.

Ground-based Measurement of Millimetre-wavelength Emission by Upper Stratospheric O₂

J. W. WATERS

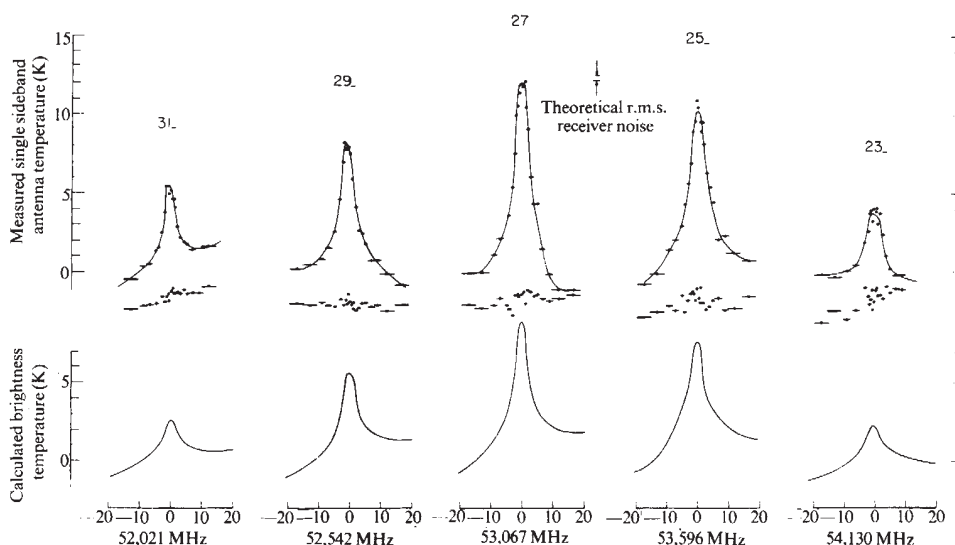
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Measurements from ground level of 53 GHz radiation from molecular oxygen in the stratosphere, using a very precise radiometer, can be used to give stratospheric temperatures.

THIS article reports measurements at sea level of upper stratospheric thermal emission from five high-rotational, millimetre-wavelength, magnetic dipole transitions of molecular oxygen, and discusses use of the emission lines for remote sensing of upper stratospheric temperatures. One of the lines, the 27-,

Molecular oxygen has a band of spectral lines near 60 GHz (5 mm wavelength) and a single line at 118 GHz produced by changes in orientation of its electronic spin relative to its rotation. The individual spin-rotation lines are designated N_+ or N_- , where N is the rotation quantum number which must be odd for $^{16}\text{O}_2$ in the $^3\Sigma_g^-$ electronic ground state, and where the subscript indicates whether the change in total angular momentum of the molecule during an emission transition is $+1$ or -1 . Each $N_{\pm}$ line has $3(2N \pm 1)$ Zeeman components spread over $\sim \pm 1$ MHz by the terrestrial magnetic field. Near the centre of the 60 GHz band the terrestrial atmosphere is quite opaque, but on the band edges thermal emission, originating in the upper stratosphere where the lines are relatively narrow, can penetrate the lower atmosphere and can be measured at the ground.

Fig. 1 Measured (upper) and calculated (lower) atmospheric zenith emission. Each measured line and the instrumental baseline shown beneath it represent integration for 16 min. The measurements were made during the week of August 30, 1972.



has been reported earlier in solar absorption¹ and in emission² but with poorer signal-to-noise and frequency-resolution than the measurements given here, and wings of some of the lines are evident in previous aircraft absorption measurements³. Stratospheric emission lines of submillimetre wavelength have also been observed recently from aircraft by a different technique^{4,5}.

The measurements reported here were made during very clear sky conditions at Haystack Observatory (elevation 0.15 km) in Westford, Massachusetts, with a radiometer constructed at the Massachusetts Institute of Technology⁶. The radiometer was a load-switched superheterodyne microwave receiver with a noise temperature of 1,800 K and no rejection of the image sideband. A linearly polarized standard-

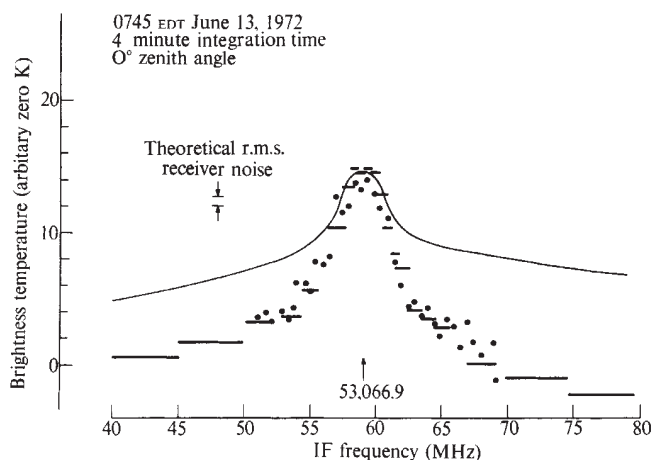


Fig. 2 Measured and calculated zenith emission from the 27₋ line. The zero levels of the measured and calculated lines are adjusted to give agreement at line centre. —, Filter system measurements; ●, haystack correlator measurements; —, calculated for 1962 US standard atmosphere.

gain microwave horn (10° beamwidth) was the antenna. The local oscillator, a klystron phase locked to a crystal reference, had frequency stability and absolute accuracy of 10^{-2} MHz. Spectral analysis was performed both by a twenty-three-channel filter bank and by the 100-channel Haystack digital autocorrelator⁷. The filter bank covered a total bandwidth of 40 MHz, with individual filters having resolutions ranging from 0.5 MHz for the centre channels to 5.0 MHz for the outer channels. Two bandwidths were used with the autocorrelator: 20 MHz with 0.5 MHz resolution, and 6.67 MHz with 0.16 MHz resolution. A small digital computer processed data from the filter bank. Data from the autocorrelator were Fourier-transformed and further processed by the Haystack computer.

Table 1 Centre Frequencies, ν_0 , and Base (—20 MHz from the Line Centre) Absolute Zenith Brightness Temperatures, T_B , for the Oxygen Lines

Transition	Measured ν_0 (MHz)	τ (nepers)	Calculated (—20 MHz from line centre)		Measured T_B , including image sideband (K)
			T_B (K)	T_B , including image sideband (K)	
23 ₋	54,130.2 ± 0.5	3.30	269	267	265 ± 2 (U)
25 ₋	53,595.9 ± 0.2	2.03	241	242	240 ± 5 (L)
27 ₋	53,066.9 ± 0.2	1.27	198	194	206 ± 10 (U)
29 ₋	52,542.4 ± 0.2	0.88	161	163	174 ± 12 (L)
31 ₋	52,021.4 ± 0.5	0.64	129	131	139 ± 15 (L)

Uncertainties in the frequencies are attributable to the uncertainties in fitting the measured emission to the calculated emission; frequencies for the 25₋, 27₋ and 29₋ lines are more accurate because they were observed with 0.16 MHz autocorrelation resolution. Uncertainties in measured absolute temperatures are attributable to the 10% calibration uncertainty. Beside the measured base temperature is indicated whether the line was observed in the upper (U) or lower (L) sideband. The zenith opacity τ calculated for the base of the lines is also given.

Figs. 1–3 show, with an increasingly expanded frequency scale, the measurements and the results of calculations. The intensities of the lines increase with frequency up to the 27₋ line because the lower rotational states have larger populations, but decrease with frequency above the 27₋ because of increasing attenuation by the lower atmosphere. A similar set of N_+ lines exists on the high-frequency band edge. The linewidths

of the measured emission correspond to pressures less than 10 mbar, proving that the emission originated at altitudes above 30 km. Table 1 gives the measured line frequencies, which agree to within measurement accuracy with the calculated values⁸ (see also refs. 9 and 10). For each line, the local oscillator frequency was shifted slightly in a separate measurement to make certain in which sideband the line appeared. The vertical axis of each figure is the brightness (equivalent blackbody radiation) temperature of the emission. Absolute accuracy of the measured amplitudes is 10% as determined by calibration of the radiometer with liquid nitrogen and thermal loads, and zero levels in the figures are arbitrary; Table 1 gives absolute values for measurements and calculations. Instrumental baselines, measured at each observed line frequency by tipping the radiometer so the horn antenna was pointed at or below the horizon, where no spectral feature is expected, are shown beneath the measured lines in Fig. 1. Fig. 2 indicates the excellent agreement between spectral analysis by the filter bank and the autocorrelator. The high-frequency resolution measurement shown in Fig. 3 represents equal observation times with the observed electric vector respectively parallel and perpendicular to the Earth's magnetic field. As expected for the latitude of the measurement, no polarization was observed.

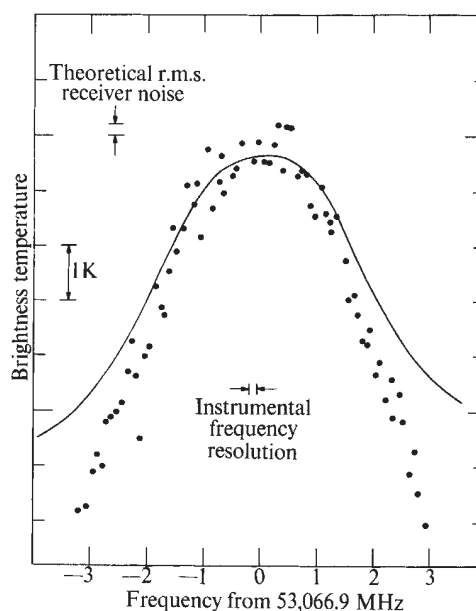


Fig. 3 High-resolution measurement and calculation of zenith emission from the 27₋ line. The measurement was made 0200–0600 EST, August 30, 1972. ●, Measured; —, calculated.

Calculations of millimetre wavelength absorption by O₂, first performed by Van Vleck¹¹, have been presented by several workers (refs. 12–17 and unpublished work of H. J. Liebe and W. M. Welch for the AGARD electromagnetic wave propagation panel). Zeeman splitting of the spin-rotation lines must be accounted for above ~45 km in the terrestrial atmosphere, where collisional broadening is sufficiently small that it does not mask the splitting. Lenoir¹² developed expressions to describe the polarized and anisotropic emission from the Zeeman components in the presence of a magnetic field and calculated emission from the mesosphere as seen from above. The calculations done here use Lenoir's matrix radiative transfer equations with the 1962 US Standard Atmosphere model¹⁸, a magnetic latitude of 55° (at which the measurements were made), and a vector dipole model of the Earth's magnetic field¹⁸. Techniques used in the calculations

are described elsewhere¹⁹. A volume mixing ratio of 0.21 is assumed for O₂ and integrations are carried to 90 km. The Gross²⁰, or Zhevakin-Naumov¹⁴, line shape is used with linewidth equal to the geometrical sum of Doppler¹² and collision¹⁷ widths. "Zero frequency", $\Delta J=0$, O₂ transitions¹¹ are included by adding their absorption to the diagonal elements of the absorption coefficient matrix. Absorption by atmospheric water vapour and other minor constituents is thought negligible (~ 0.05 neper) and is not included in the calculations.

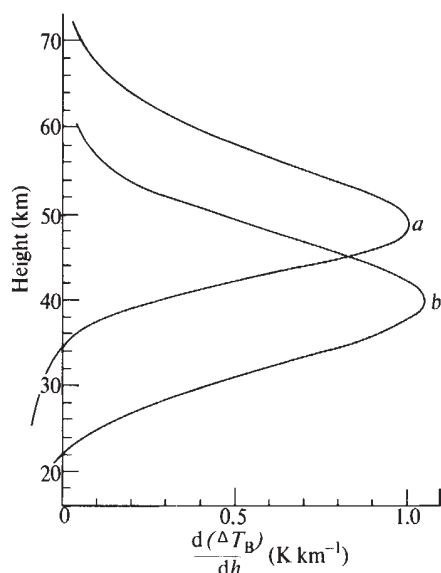


Fig. 4 Height resolution for ground-based sensing of upper stratospheric temperatures from O₂ millimetre-wavelength emission. a, $\nu_2 = \nu_0 = 53,066.9$ MHz, $\nu_1 = \nu_0 - 2.5$ MHz; b, $\nu_2 = \nu_0 - 2.5$ MHz, $\nu_1 = \nu_0 - 20$ MHz.

The discrepancy between measured and calculated line amplitudes is significantly larger than the radiometer calibration uncertainty. The image sideband contributes a slope to the measured lines, but cannot account for the amplitude disagreement. Atmospheric temperatures must be warmer than the model used for calculations by ~ 50 K at altitudes of 30–60 km in order to explain the discrepancy, a highly unlikely situation for the time and location of the measurements²¹, and reported anomalous O₂ concentrations²² occur at altitudes too high to contribute significantly to the emission measured here. The discrepancy probably arises from the theory used for the calculations, which sums the (assumed incoherent) absorption of the individual lines. A theory which accounts for coherence in overlapping lines has been applied²³ to absorption by pure O₂ at a pressure of several atmospheres and gives better agreement with measurement than calculations which simply sum individual line absorption. This theory has also been used²⁴ to calculate O₂ absorption at atmospheric pressures, but quantitative comparisons with measurement have not yet been made.

Previous publications have described how millimetre-wavelength O₂ emission can be used to sense temperatures up through the mesosphere from satellite-based measurements^{12,13,16,27} and tropospheric temperatures from ground-based measurements^{25–27}. Temperatures in the stratosphere can also be sensed from ground-based measurements of the O₂ emission lines reported here. High altitude emission is distinguishable from low altitude emission because of the strong dependence of linewidth on altitude up to ~ 50 km where Zeeman splitting produces an effective linewidth that is only a weak function of altitude. Emission above ~ 70 km

contributes negligibly to the lines because the absorption coefficient at line centre is then proportional to O₂ density, not to mixing ratio as when collisions dominate line broadening, and line emission decreases exponentially with altitude. Variations in absorption by the lower atmosphere can be corrected for by measurements of the base of the line. Fig. 4 shows the altitude region sensed by the ground-based measurements, where the two curves give as a function of altitude the contribution per unit altitude to the measured emission at the ground. The curve with the higher peak is for the difference in measured emission between the centre of the line and 2.5 MHz away from the centre; that with the lower peak is for the difference in measured emission between 2.5 MHz and 20 MHz from the centre of the line. The high rotational lines are sensitive functions of temperature; a 1% change in upper stratospheric temperature produces a 5% change in emission at the centre of the 27_– line². The instrument described here can measure upper stratospheric temperature variations with r.m.s. accuracies of ~ 1 K for an integration time of 1 h. Stratospheric phenomena which can be conveniently studied from the ground by this measurement technique include diurnal temperature variation²⁸ and sudden warmings²⁹.

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- ¹ Kahan, W., *Nature*, **195**, 30 (1962).
- ² Waters, J. W., *Proc. Seventh Inter. Symp. Remote Sensing of Environment*, Univ. of Mich. (1971).
- ³ Carter, C. J., Mitchell, R. L., and Reber, E. E., *J. Geophys. Res.*, **73**, 3113 (1968).
- ⁴ Harries, J. E., Swann, N. R. W., Beckman, J. E., and Ade, P. A. R., *Nature*, **236**, 159 (1972).
- ⁵ Nolt, I. G., Radostitz, J. V., and Donnelly, R. J., *Nature*, **236**, 444 (1972).
- ⁶ Waters, J. W., Paroskie, R. M., Barrett, J. W., Papa, D. C., and Staelin, D. H., *Res. Lab. Elec., Q. Progress Rpt. 107*, 23 (Massachusetts Institute of Technology, 1972).
- ⁷ Weinreb, S., *Res. Lab. Elec. Tech. Rpt.*, 412 (Massachusetts Institute of Technology, 1963).
- ⁸ Wilheit, jun., T. T., thesis, Massachusetts Institute of Technology (1969).
- ⁹ Welch, W. M., and Mizushima, M., *Phys. Rev., A*, **5**, 2692 (1972).
- ¹⁰ Wilheit, jun., T. T., and Barrett, A. H., *Phys. Rev., A*, **1**, 213 (1970).
- ¹¹ Van Vleck, J. H., *Phys. Rev.*, **71**, 413 (1947).
- ¹² Lenoir, W. B., *J. Geophys. Res.*, **73**, 361 (1968).
- ¹³ Meeks, M. L., and Lilley, A. E., *J. Geophys. Res.*, **68**, 1653 (1963).
- ¹⁴ Zhevakin, C. A., and Naumov, A. P., *Izvest. VUZ Radiofiz.*, **9**, 433 (1966).
- ¹⁵ Reber, E. E., Mitchell, R. L., and Carter, C. J., *IEEE Trans. Ant. and Prop.*, **AP-18**, 472 (1970).
- ¹⁶ Croom, D. L., *Planet. Space Sci.*, **19**, 777 (1971).
- ¹⁷ Reber, E. E., *J. Geophys. Res.*, **77**, 3831 (1972).
- ¹⁸ Valley, S. L., ed., *Handbook of Geophysics and Space Environments* (McGraw-Hill, New York, 1965).
- ¹⁹ Waters, J. W., thesis, Massachusetts Institute of Technology (1970).
- ²⁰ Gross, E. P., *Phys. Rev.*, **97**, 395 (1955).
- ²¹ Cole, A. E., *Space Research*, **11**, 813 (Akademie-Verlag, Berlin, 1971).
- ²² Tchen, C. M., *Space Research*, **11**, 899 (Akademie-Verlag, Berlin, 1971).
- ²³ Gordon, R. G., *J. Chem. Phys.*, **46**, 448 (1967).
- ²⁴ Mingelgrin, U., *US Dept. of Commerce Telecommunications Res. and Eng. Rep.*, 32 (1972).
- ²⁵ Westwater, E. R., and Strand, O. N., *Tech. Rept. IER37-ITSA37* (US Dept. of Commerce, 1967).
- ²⁶ Miner, G. F., Thornton, D. D., and Welch, W. J., *J. Geophys. Res.*, **77**, 975 (1972).
- ²⁷ Staelin, D. H., *Proc. IEEE*, **57**, 427 (1969).
- ²⁸ Groves, G. V., *IQSY 5*, Paper 14 (1969).
- ²⁹ Barnett, J. J., Harwood, R. S., Houghton, J. T., Morgan, C. G., Rodgers, C. D., Williamson, E. J., Peckham, G., and Smith, S. D., *Nature*, **230**, 47 (1971).

Genetic Control of Mitochondrial Enzymes in Human-Mouse Somatic Cell Hybrids

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The two techniques for detecting human enzymes in man/mouse cell hybrids described here provide a promising and interesting approach to the mapping of chromosomal genes coding for mitochondrial enzymes. The authors confirm the nuclear gene specification of citrate synthase and malate dehydrogenase, and give information on their possible linkage with each other and with other genetic markers.

MITOCHONDRIAL biogenesis depends on an interaction between the genetic activities of nuclear and mitochondrial DNA. The study of this interaction ideally requires a genetic system in which both chromosomal and mitochondrial segregation can be observed. Until recently these requirements were fulfilled chiefly by the lower eukaryotes: yeasts¹, *Neurospora*², *Paramecium*³ and *Tetrahymena*⁴. Here we describe the first results of a coordinated study of mitochondrial biogenesis using somatic cell hybrids.

Somatic cell hybrids, especially man-mouse and man-Chinese hamster, have been used to map human chromosomes^{5,6}. The presence of human enzymes, or non-enzyme proteins, which are electrophoretically distinguishable from their mouse or hamster homologues, can be correlated with the presence of other enzymes or proteins and specific chromosomes after the loss of human chromosomes. Theoretically, segregation of the mitochondria of the two parent species could be observed as well as interaction of the organelle of one species with at least some nuclear coded products of the other.

We have examined nine independently isolated man-mouse hybrid lines for the presence of two mitochondrially located⁷ human enzymes that are usually assumed to be nuclear coded: NAD malate dehydrogenase (E.C. 1.1.1.37) and citrate synthase (E.C. 4.1.3.7). Where the usual electrophoretic methods could not separate human from mouse enzymes, immunological techniques were used to identify the human enzymes. Our studies confirm the nuclear control of these enzymes and give information on their possible linkage with each other, and with other genetic markers. The hybrids were also examined for the presence of a large number of other human enzymes (our unpublished results obtained in collaboration with H. Harris, D. Hopkinson, S. Povey and their colleagues).

Two groups^{8,9} have shown that all the human-mouse hybrid lines examined by them (ten in all) had apparently lost the

human mitochondrial DNA, whilst retaining the mouse component. Three of the hybrid lines we analysed were amongst those shown by Clayton *et al.*⁸ to have no human mitochondrial DNA detectable by CsCl centrifugation.

Citrate synthase (CS) and NAD-malate dehydrogenase (MOR) are consecutively acting enzymes of the citric acid cycle, found in the mitochondrial matrix⁷. CS is thought to occur only in mitochondria, whereas MOR has a cytoplasmic counterpart (sMOR) which is apparently coded for at an independent nuclear locus^{10,11}.

Cytoplasmic sMOR is easily separated by electrophoresis from mitochondrial mMOR in both mouse and man, and the sMOR of the two species is also electrophoretically distinguishable (Fig. 1). Citrate synthase from the two species can now also be resolved by electrophoresis (as found by Craig²⁴) on 'Cellogel' strips (Reeve Angel cellulose acetate).

We have not been able to separate mouse from human mMOR by electrophoresis in any of several buffer systems between pH 5.2 and 9.0 on polyacrylamide, starch or 'Cellogel'. This same problem has also arisen with a number of other mitochondrial enzymes, as discussed later, and has forced us to consider other approaches to distinguishing human and mouse enzymes.

One approach to this problem would be the analysis of hybrids involving one parent which is either heterozygous or homozygous for an electrophoretically mutant form of mMOR. Such mutants, however, are not available in mouse inbred strains¹² and thus, so far, in cell lines; and in a human population studied by Davidson and Cortner¹⁰ the frequency of heterozygotes for an electrophoretic variant of mMOR was only 1%.

Immunological Approach

The use of immunological methods to distinguish human and mouse enzymes and other proteins provides another general approach to the study of human markers.

Specific antisera to human proteins or enzymes that do not cross-react with the mouse counterparts of these proteins can be obtained by immunizing mice with the appropriate human material. In the case of enzymes, these sera can be used for identification of human enzyme by immunodiffusion or immunoelectrophoresis followed by specific staining for the relevant enzyme. This procedure has been used to screen hybrids for the presence of human mMOR.

Human mMOR was purified from placental mitochondria prepared essentially by the method of Freeman¹³ but omitting the sucrose gradient step. The mitochondria from a 1 kg placenta, suspended in 7.0 ml. 0.3 M sucrose-0.002 M EDTA-0.03 M nicotinamide-0.5% 'Triton' X-100, were sonicated for 4 × 30 s. The sonicate was spun and the supernatant layered onto a

5 × 15 cm column of 'Amberlite' CG50 equilibrated with 0.025 M sodium phosphate buffer pH 7.0 (ref. 14). The column was washed with 300 ml. of the equilibrating buffer to remove contaminating sMOR activity, and then with 600 ml. of 0.2 M sodium phosphate pH 7.0 to elute the mMOR. Fractions with malate dehydrogenase activity, assayed as described by England and Siegel¹⁵, were pooled and concentrated by Amicon ultrafiltration. The resulting sample, which was free of sMOR and contained 300 µg protein ml.⁻¹ and 30 units¹⁵ per mg protein of malate dehydrogenase activity, was used as the immunizing antigen.

A sample of human sMOR was prepared by the same ion exchange procedure using the supernatant fractions from the same placental preparation.

As the mouse parent in most cell hybrids examined was an 8-azaguanine resistant L cell (1R)¹⁶ immunization was carried out in C₃H mice, the inbred line from which L cells were derived. The first subcutaneous injection into four sites was 0.4 ml. of 1 : 1 v/v emulsion of antigen with Freund's complete adjuvant. Four weeks later, and subsequently at intervals of 1 week, booster injections were given in the same manner but using incomplete adjuvant for the emulsification. Immediately before each boost the mice were bled from the tail and serum was prepared. The sera were titred by double diffusion in agar against the original antigen. When a good titre was attained, the mouse was given an intraperitoneal injection of antigen and 10 × 10⁶ live sarcoma S180 cells¹⁷. The relatively large volume of ascites fluid, produced within 7–10 days in response to the sarcoma, was of lower titre but yielded greater total antibody activity (by about a factor of 5) than could the available serum. When necessary, the sarcoma fluid was concentrated by dialysis against 18% sodium sulphate for 8 h, dissolving the precipitate formed in water, and dialysing at 4° C against phosphate buffered saline. Sera from different mice or different bleedings were not pooled.

Hybrid cells were grown in 'Biocult' RPMI 1640 medium supplemented with 10% foetal calf serum and the components of Littlefield's HAT selective medium, hypoxanthine, thymidine and amethopterin¹⁶. Mouse and human cell lines were grown in the same medium but without the HAT components.

Cell extracts were made by sonicating 40–50 × 10⁶ cells in 0.45 ml. lysis buffer (5 × 10⁻² M phosphate, 1 × 10⁻³ M mercaptoethanol, 2 × 10⁻⁵ M NADP, pH 7.0) for 10 s. The sonicates were clarified by a 1-min spin in a Beckman 'Microfuge' and stored in aliquots in liquid nitrogen. Their total malate dehydrogenase activity was measured spectrophotometrically¹⁵ and adjusted to comparable levels where necessary.

Enzyme Activity of Hybrid Lines

The hybrid lines whose parentage is described in Table 1 were examined for: (i) citrate synthase activity by electrophoresis on 'Cello-gel' (recently found by Craig²⁴); (ii) NAD-malate dehydrogenase activity, also by electrophoresis; (iii) human mMOR activity by immunodiffusion using specific antiserum; (iv) a large number of other enzymes by electrophoresis on starch (our unpublished results obtained in collaboration with H. Harris, D. Hopkinson, S. Povey and their colleagues); and (v) chromosomes.

The electrophoretic pattern obtained for citrate synthase using extracts from some of the hybrid lines is shown in Fig. 2. In addition to the mouse citrate synthase band, which is seen in all the lines, some hybrids have another band of activity intermediate in mobility between that shown by the two parental lines. This intermediate band most probably represents a heteropolymeric molecule (A^M A^H) produced by the random interaction, in proportion to their concentrations, of mouse (A^M) and human (A^H) subunits. These patterns were consistent and reproducible in repeated runs with extracts from the same cell lines. The lesser staining activity of the hybrid band may be caused by reduced activity of the heteropolymeric enzyme in the hybrid cell or, more likely, unequal

production of human and mouse subunits because of a gene dosage effect. Such unequal production is most simply explained by extensive human chromosome loss during the evolution of the hybrid lines. This will usually leave only one copy of the human gene in the hybrid whilst most if not all of the heteroploid mouse parent's chromosomes will be retained, possibly leaving several copies of the corresponding mouse gene. Similar results have been observed before for other enzymes¹⁸.

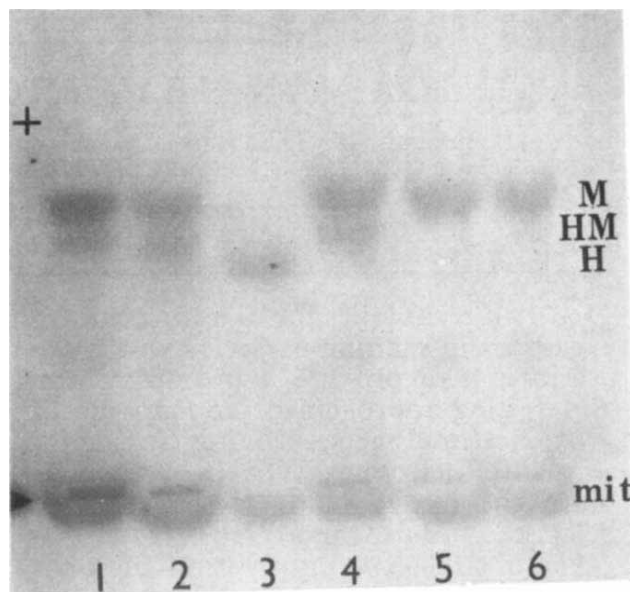


Fig. 1 NAD-malate dehydrogenase electrophoresis on 'Cello-gel' (photograph of gel); 0.5 µl. samples were applied; the gel was run in 2.8 M sodium citrate–17.2 mM disodium hydrogen phosphate pH 7.0 buffer for 1.25 h at 200 V at room temperature. Stain: Total volume 2 ml. including 0.4 ml. 1 M Tris-HCl pH 8.0; 0.2 ml. 0.85 M malate pH 7.5; 10 µl. 0.05 M NAD⁺; 50 µl. 1% thiazolyl blue (MTT); two crystals phenazine methosulphate (PMS). Identification of samples: 1, 4.42Z; 2, 4.12Z; 3, BREL; 4, HORP 3.2.1; 5, 1W1; 6, 1R. The arrow indicates the origin, and mit the mitochondrial enzyme band. H, HM and M refer respectively, to the human, human-mouse heteropolymer and mouse cytoplasmic forms.

An electrophoretogram stained for the MOR activity of extracts from some hybrids and controls is shown in Fig. 1. Mouse, human and hybrid extracts show indistinguishable cathodally migrating mMOR bands even when run for much longer times than shown. As has been reported before, however, by a number of workers^{18,19}, human and mouse sMOR can be resolved. Amongst the hybrids three have human as well as mouse cytoplasmic enzyme activity, again manifested only by a heteropolymeric band of intermediate mobility. As expected, all the hybrid lines examined show mouse sMOR activity.

Mitochondrial malate dehydrogenase was sought in the hybrid cell extracts by Ouchterlony double diffusion in agar, using the antisera produced in mice, as described above. Precipitin lines were visualized by staining for malate dehydrogenase activity using a tetrazolium system (Fig. 3). As expected after the precautions taken with the immunization procedure, and from published work on the relationship between sMOR and mMOR²⁰, no precipitin reaction was observed with the mouse lines 1R or 3T3, nor with human sMOR. The partially purified human mMOR (the immunizing antigen) gave a single, completely continuous precipitin line with the human lymphocyte line BREL (derived from the human parent in Z hybrids (Table 1, ref. 18)). No cross-reaction was found with a whole range of dilutions of commercial pig heart mMOR. This

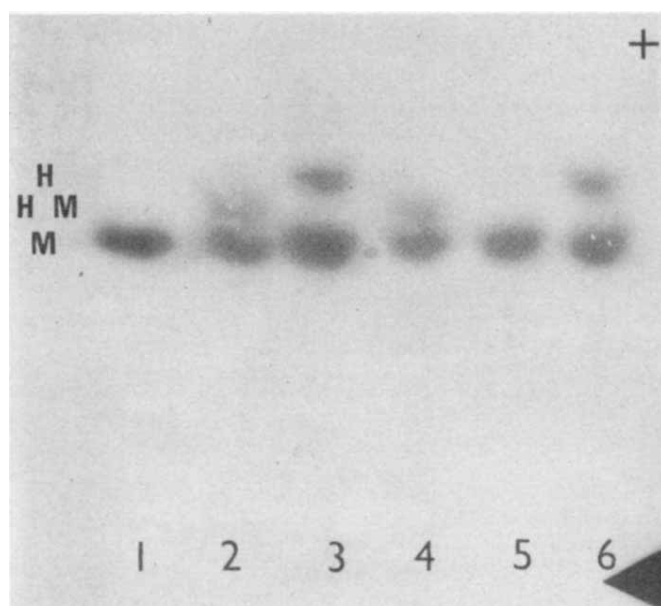


Fig. 2 Citrate synthase electrophoresis on 'Cellogel' (photograph of gel); 0.5 μ l. samples were applied to 'Cellogel' strips and electrophoretic separation carried out in 0.1 M Tris adjusted to pH 8.0 with 0.1 M NaH_2PO_4 , for 2 h at 150 V at room temperature. The staining mixture based on the reduction of dichlorophenol indophenol by reduced coenzyme A is to be reported elsewhere²⁴. Identification of samples: 1, 4W10; 2, 3W4; 3, 1R+BREL; 4, HOPR 3.2.1; 5, HORL 4.1; 6, 1R+BREL.

may mean that the precaution of immunizing mice to avoid cross-reaction is not necessary with this particular enzyme.

Lactate dehydrogenase (LDH) patterns were established by electrophoresis on 'Cellogel'⁶. The presence of human A and B subunits (the latter on the basis of quantitative differences only) was scored as described previously¹⁸.

Table 1

Hybrid lines	Human parent	Mouse parent
1W1, 2W1, 3W4, 4W10 (ref. 16)	Normal lymphocytes (MAR)	1R, 8-azaguanine resistant L-cell
HORL 4.1, HOPR 3.2.1	Normal lymphocytes (HOW)	1R, 8-azaguanine resistant L-cell
Not previously examined, made as in ref. 16		
4.12Z, 4.31Z, 4.42.7Z (ref. 18)	Normal lymphocytes (BRE)	1T, bromodeoxyuridine resistant subclone of 3T3

The combined results of repeated testing of the nine hybrids for CS, mMOR, sMOR and LDHB—also peptidase B (our unpublished results obtained in collaboration with H. Harris, D. Hopkinson, S. Povey and their colleagues) because of linkage—are shown in Table 2, together with information on the presence of mitochondrial DNA where this is available.

Implications

Three principal implications for the control of the two mitochondrial enzymes are suggested by the data in Table 2. First, of the hybrid lines shown by CsCl isopycnic centrifugation⁸ to have no human mitochondrial DNA, three were also studied by us. Of these, two have both the mitochondrial enzymes studied and one has neither. Thus we can conclude

that the presence of human mitochondrial DNA is not required for the expression of at least some human mitochondrial functions. Whether this is because there is no strong control by mitochondrial DNA on all the nuclear coded products which eventually become incorporated into the organelle, or because such control exists² but is not species specific, we cannot say on the basis of present data.

An interesting question which arises is whether the human nuclear coded mitochondrial enzymes produced in these hybrids are in fact located in the mitochondria which are presumed to contain only mouse mitochondrial DNA. The fact that a heteropolymeric band of citrate synthase is observed on electrophoresis of certain hybrids, but not when a mixture of human and mouse cell extracts is run together²⁴, suggests that in some location in the hybrid cell the human and mouse enzymes are found together. Supporting this deduction, preliminary results from immunodiffusion with sonicated mitochondria from sucrose gradient fractionation¹³ of large quantities of 2W1 show that human mitochondrial malate dehydrogenase activity is located in the mitochondria of this hybrid line.

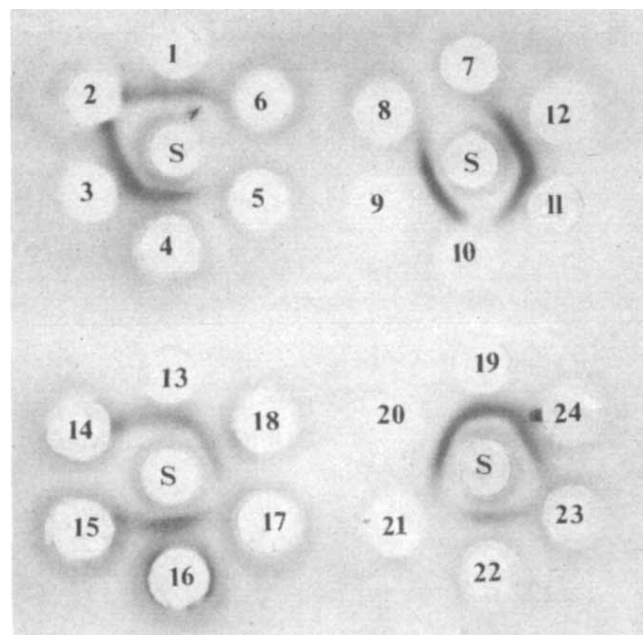


Fig. 3 Ouchterlony double diffusion in agar, using the specific mouse anti-human mMOR serum, stained for malate dehydrogenase activity. Supporting medium: 1.5% Nobel agar in 0.9% NaCl solution. 6 μ l. cell extract per well used. Diffusion took place for 36 h at 4° C. Slides were washed in 0.9% aqueous NaCl for 24 h at 4° C. Staining as for 'Cellogel'. S in the centre well refers to the antibody containing sarcoma induced ascites fluid. Identification of other wells: 1, BREL (38.7); 2, sMOR; 3, mMOR immunizing antigen (59.7); 4, 2W1 (139.8); 5, 1R (212.4); 6, 1W1 (120.6); 7, 1R; 8, HORL 4.1 (147.0); 9, mMOR; 10, 4W10 (138.6); 11, BREL; 12, 3W4 (139.8); 13, BREL; 14, 4.42Z (140.4); 15, 3T3 (179.4); 16, 4.31Z (166.8); 17, 3T3; 18, 4.12Z (141.6); 19, BREL; 20, mMOR; 21, sMOR; 22, HOPR 3.2.1 (127.2); 23, 1R; 24, HOPR 3.2.1. Numbers in brackets give malate dehydrogenase activities in 6 μ l. of cell extract, in units $\times 10^3$ (ref. 15).

The second feature of the data is the absence of a specific relationship between the expression of cytoplasmic and mitochondrial malate dehydrogenase in the hybrid cells examined. This is in agreement with genetic data on mutant forms of both enzymes in man and mouse¹⁰⁻¹². It is worth mentioning that the three hybrids that have sMOR activities are also the only ones showing cytoplasmic NADP isocitrate dehydrogenase activity, an association that is consistent with the suggested linkage between these two functions²¹.

The third and perhaps the most striking feature of the data of Table 2 is that there seems to be a strong positive correlation between the presence of human citrate synthase and mitochondrial malate dehydrogenase in the hybrid lines examined. The unrelated enzyme functions LDHB and peptidase B, the loci for which are linked to each other and are probably on chromosome 12 (ref. 19), also show a marked positive correlation with the expression of CS and mMOR. In fact, the only exception to a complete correlation between the presence and absence of these four enzymes, which is expected if they are all controlled by genes on the same chromosome, is the hybrid line 2W1. None of the other 28 enzyme activities tested for (our unpublished results obtained in collaboration with H. Harris, D. Hopkinson, S. Povey and their colleagues) showed any obvious association with any of these four enzymes. Of the total of thirty-two enzyme activities that were studied, 2W1 shows only mMOR and the X-linked activities hypoxanthine guanine phosphoribosyltransferase (HGPRT), phosphoglycerate kinase (PGK) and glucose-6-phosphate dehydrogenase (G6PD). These X-linked functions are expected to be present because the human X chromosome is selectively retained in these hybrids in the HAT medium. It is tempting to suggest that the four enzyme CS, mMOR, LDHB and peptidase B are all controlled by genes on the same chromosome, presumably number 12, and that 2W1 contains only a fragment of this chromosome perhaps, for example, just one arm. In this case this fragment must carry the gene for mMOR but not the other three genes. A complete karyotypic study of 2W1 is under way, but preliminary observations do suggest that 2W1 retains perhaps at most one recognizable human chromosome other than the X.

Table 2 Mitochondrial Enzyme and Other Activities in Nine Different Human-Mouse Hybrids

Hybrid cell line	CS	mMOR	sMOR	mitDNA	LDHB/pepB
1W1	—	—	—	NT	—
2W1	—	+	—	NT	—
3W4	+	+	—	—	+
4W10	—	—	—	—	—
HORL 4.1	—	—	—	NT	—
HORP 3.2.1	+	+	+	NT	+
4.12Z	±*	+	+	—	+
4.31Z	+	+	—	NT	+
4.42.7Z	—	—	+	NT	—

+, Presence of human activity; —, absence; NT, not tested.

*, Very weak activity possibly because of an extreme gene dosage imbalance, as 4.12Z has a doubled chromosome complement from its mouse parent, 3T3.

CS, Citrate synthase; mMOR, sMOR are respectively mitochondrial and cytoplasmic NAD-malate dehydrogenase; mitDNA, mitochondrial DNA; LDHB, lactate dehydrogenase subunit B; pepB, peptidase B.

Only the presence of human activities and mitDNA in the hybrid lines is recorded in this table. In all cases, however (except for mMOR), the mouse component is invariably present. In the case of mMOR the immunological technique used so far scores only for the human activity.

In the course of screening electrophoretic properties of mitochondrial enzymes the difficulty of separating mouse and human forms in the case of several enzymes was striking. Others have encountered similar difficulties (refs. 6, 10 and our unpublished results obtained in collaboration with H. Harris, D. Hopkinson, S. Povey and their colleagues). We find that the mitochondrial component at around physiological pH migrates only a short distance to one or other side of the origin, and is always more cathodal than the cytoplasmic enzyme.

Whether because of these characteristics, or because there is a strong selective conservation of charge properties amongst these enzymes (MOR, GOT (glutamate oxaloacetate transaminase), IDH (NADP isocitrate dehydrogenase)), it is difficult if not impossible to separate mouse and human components sufficiently to allow hybrid analysis by electrophoretic means. The immunological approach may therefore find more widespread use in these obstinate cases. Conservation of charge amongst these mitochondrial enzymes is perhaps also suggested by the low frequency of electrophoretic variants in the populations so far examined²². It is not impossible that the similar and apparently invariable charge of these enzymes at physiological pH values is a requirement for their localization in mitochondria.

There are exceptions to this suggested charge conservation. Mitochondrial NADP malate dehydrogenase migrates to the anode in the mouse and to the cathode in man at pH 7.0^{12,23}. But this enzyme shows a high frequency of polymorphism (30% heterozygotes for the variant) in the populations that have been examined²³.

The results of this study demonstrate once more the power of somatic cell hybridization as a tool for a combined genetic and biochemical analysis of eukaryotic systems. We have confirmed the nuclear gene specification of two mitochondrial enzymes, citrate synthase and malate dehydrogenase, and established a possible linkage between them and with the enzymes LDH-B and peptidase B on chromosome 12. We have also confirmed the genetic independence of the mitochondrial and supernatant forms of malate dehydrogenase. If our genetic interpretation is correct, then it is probable that the genes for CS and mMOR are not clearly linked, so that this linkage may have no special functional significance. Further work is in progress to extend this analysis to other mitochondrial enzymes, and to proteins and other gene products that are likely to be coded for by the mitochondrial DNA.

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- Schatz, G., Mason, T., Ebner, E., and Poyton, R. O., *Abstr. Commun. Meet. Fed. Eur. Biochem. Soc.*, **8**, Abstr. No. 564 (1972).
- Barath, Z., and Kuntzel, K., *Proc. US Nat. Acad. Sci.*, **69**, 1371 (1972).
- Adoutte, A., and Beisson, J., *Nature*, **235**, 393 (1972).
- Borst, P., *Ann. Rev. Biochem.*, **41**, 333 (1972).
- Bodmer, W. F., *Proc. Fourth Int. Cong. Genet., Paris* (in the press).
- Meera Khan, P., *Arch. Biochem. Biophys.*, **145**, 470 (1971).
- Ashwell, M., and Work, T. S., *Ann. Rev. Biochem.*, **39**, 251 (1971).
- Clayton, D. A., Teplitz, R. L., Nabholz, M., Dovey, H., and Bodmer, W., *Nature*, **234**, 560 (1971).
- Attardi, B., and Attardi, G., *Proc. US Nat. Acad. Sci.*, **69**, 129 (1972).
- Davidson, R., and Cortner, J., *Science*, **157**, 1569 (1967).
- Davidson, R., and Cortner, J., *Nature*, **215**, 761 (1971).
- Shows, J. B., Chapman, N. M., and Ruddle, F. H., *Biochem. Genet.*, **4**, 707 (1970).
- Freeman, K. B., *Biochem. J.*, **94**, 494 (1965).
- Thorne, C. J. R., and Dent, N. J., *FEBS Symp.*, **18**, 203 (1970).
- Englard, S., and Siegel, L., *Methods in Enzymology*, **13**, 99 (1969).
- Nabholz, M., Miggiano, V., and Bodmer, W., *Nature*, **223**, 358 (1969).
- Herrmann, E. C., and Engle, C., *Proc. Soc. Exp. Biol. Med.*, **98**, 257 (1958).
- Santachiara, A. S., Nabholz, M., Miggiano, V., Darlington, A. J., and Bodmer, W., *Nature*, **227**, 248 (1970).
- Ruddle, F. H., in *Advances in the Biosciences*, **8**, 299 (1972). Workshop on Mechanisms and Prospects of Genetic Exchange (Pergamon).
- Kitto, G. B., and Kaplan, N. O., *Biochemistry*, **5**, 3966 (1966).
- Shows, T. B., *Biochem. Genet.*, **7**, 193 (1972).
- Harris, H., and Hopkinson, D. A., *Ann. Hum. Genet.*, **36**, 9 (1972).
- Cohen, P. T., and Omenn, G. S., *Biochem. Genet.*, **7**, 303 (1972).
- Craig, I., *Biochem. Genet.* (in the press).

LETTERS TO NATURE

PHYSICAL SCIENCES

New Kind of Ring Around Saturn?

TITAN, a satellite of Saturn, is the only satellite in the Solar System known to possess an atmosphere. That such a small body, only 87% more massive than the Earth's Moon and with a diameter but 40% larger¹, should have an atmosphere is a mystery to which Sagan² may have provided an answer—outgassing of the satellite. But barring extraordinary circumstances, the satellite must lose its atmosphere at a prodigious rate. But what happens to the atmosphere after it leaves Titan?

What is remarkable about the ejected atmosphere is that, although it has Titanian escape velocity, it probably does not have Saturnian escape velocity. Trafton³ has estimated the exospheric temperature of Titan to be 74 K. At that temperature, even hydrogen atoms have an r.m.s. thermal speed of only 1.4 km s⁻¹. Because Titan's orbital speed about Saturn is 5.6 km s⁻¹, the average ejected hydrogen atom will not reach the Saturnian escape velocity of 7.9 km s⁻¹ (at Titan's orbit) even if ejected forwards. Consequently, atoms and molecules lost by Titan are forced by the planet's gravitational field to orbit Saturn until ionized or until they are recaptured by Titan, forming a gaseous torus encompassing Titan's orbit. Once ionized, they will be swept away by the solar wind, unless Saturn has a magnetosphere extending to Titan's orbit.

The composition of the Titanian atmosphere is believed²⁻⁵ to be either predominantly molecular hydrogen or a mixture of comparable amounts of hydrogen and methane. We assume the ejected hydrogen to be photodissociated into atomic hydrogen. The lifetime of an atom of hydrogen subject to solar wind charge-exchange ionization is about 6 yr or 140 Titanian orbital periods at the distance of Saturn⁶⁻⁸. The solar photoionization lifetime will be even longer⁹. Consequently, the torus can store a 6-yr supply of Titanian hydrogen. For a temperature of ~70 K, the torus will have a width, in the equatorial plane, of the order of the orbital radius of Titan, r_T , which is 20 Saturnian radii, and a thickness, normal to the plane, of $\sim r_T/2$. We take the volume of the torus to be $\sim r_T^3$, and use Trafton's Titanian escape rates³ (2×10^{26} to 4×10^{29} molecules s⁻¹); we find that the density of the torus is ~ 40 to $\sim 9 \times 10^4$ atoms cm⁻³. A torus of these densities would probably be detectable from Earth orbit by resonantly scattered Lyman- α radiation, or by absorption during an occultation by the torus of an interstellar Lyman- α source. If Saturn has a magnetosphere, however, the density could be smaller because of charge exchange with magnetospheric plasma.

We estimate that, if the Titanian exospheric radius is the order of 10 Titanian radii, as in some of Trafton's models³, more than 90% of the escaping atoms could be recaptured by Titan (which lowers the upper limit of toroidal density to $\sim 2 \times 10^3$ cm⁻³). Hence, the torus presents a mechanism which allows satellites previously thought incapable of possessing an atmosphere to have one, and it suggests a sensitive way of testing for atmospheres of distant satellites by Lyman- α observations. The outer planets, where the solar ionization rate is small, are most conducive to the existence of such gaseous toruses. A more detailed study of this problem is

given by T. R. McD. and N. M. B. (to be published). Saturn, in particular, should be examined for a gaseous torus that is larger, in absolute dimensions, than the Sun.

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- ¹ Newburn, jun., R. L., and Gulkis, S., *JPL Technical Report 32-1529* (Jet Propulsion Laboratory, Pasadena, Calif., 1971).
- ² Sagan, C., *Icarus* (in the press).
- ³ Trafton, L., *Astrophys. J.*, **175**, 285 (1972).
- ⁴ Kuiper, G. P., *Astrophys. J.*, **100**, 378 (1944).
- ⁵ Pollack, J. B., *Icarus* (in the press).
- ⁶ Semar, C. L., *J. Geophys. Res.*, **75**, 6892 (1970). [Erratum in *J. Geophys. Res.*, **76**, 5374 (1971).]
- ⁷ McDonough, T. R., and Brice, N. M., *Icarus*, **15**, 505 (1971).
- ⁸ Axford, W. L., *Solar Wind* (edit. by Sonett, C. P., Coleman, jun., P. J., and Wilcox, J. M.) NASA No. SP-308 (US Govt Printing Office, Washington, DC, 1972).
- ⁹ Hinteregger, M. E., *Ann. Geophys.*, **26**, 547 (1970).

Apollo 17 Age Determinations

THE Apollo 17 mission landed in a valley in the highland region forming part of the south-eastern rim of the Sea of Serenity (20° 09' 50" N, 30° 44' 58" E). An early allocation of a limited number of samples has been made to provide preliminary information on this site and we report here the results of age determinations we have made on two crystalline rocks using the ⁴⁰Ar-³⁹Ar and ³⁸Ar-³⁷Ar dating techniques^{1,2}. The description of sample documentation and relationship of samples to the geology at the Apollo 17 site is based on a preliminary report (Astrogeology 71) issued by the Apollo Lunar Geology Investigation team and may be subject to modification. Sample classification is based on information supplied by the Preliminary Examination team at the Manned Spacecraft Center.

The samples analysed were: (1) A coarse grained basalt, 75055, which was part of a rock fragment removed by astronaut Schmitt from a metre sized boulder on the rim of Camelot crater (station 5). 75055 consists of approximately 35% plagioclase, 45% yellowish brown pyroxene and 20% opaque minerals (ilmenite). The dominant grain size was around 1 mm and in the hand specimen the sample resembled the Apollo 11 low-K basalts. Preliminary examination of the samples from the vicinity of Camelot crater indicates that subfloor basalts covered this part of the valley floor to a depth of at least 100 m before formation of the crater. 75055 may be regarded as representative of this subfloor basalt unit. We have analysed a 50 mg rock chip of 75055. (2) A sample, 76055, described in the preliminary examination as a vesicular anorthositic gabbro. This sample was collected by the astronauts 3 km north of the

landing site, at station 6, near the base of the large mountain referred to as the North Massif. The anorthositic gabbro seems to be a principal rock type at station 6 forming a matrix to other types of brecciated samples. Preliminary examination at Houston indicates that the material is thoroughly recrystallized. Our sample of 76055 consisted largely of a greenish-grey fine grained matrix of plagioclase and smaller amounts of pyroxene with irregular lenses of pyroxene and plagioclase which appear in binocular microscope examination of the hand specimen to be a coarser grained version of the matrix. We have analysed a 70 mg chip of the matrix and a 19 mg chip of one of the lens-like clasts. At present we do not have a thin section description of either sample.

The samples were vacuum encapsulated in quartz ampoules and irradiated in the core of the Herald reactor, AWRE, Aldermaston. Three samples of a terrestrial hornblende Hb3 gr, described previously², were spaced around the lunar samples to monitor the conversion of ^{39}K to ^{39}Ar . ($^{40}\text{Ar}/^{39}\text{Ar}$) ratios, corrected for atmospheric ^{40}Ar and calcium derived ^{39}Ar , were measured for argon extracted from the three monitors as 15.11 ± 0.09 , 15.04 ± 0.04 , and 15.12 ± 0.06 . The irradiation parameter, $J = (\exp(\lambda T) - 1) / (^{40}\text{Ar}/^{39}\text{Ar})$, was thereby determined as 0.0501 ± 0.0010 ($T = 1.062 \pm 0.020 \times 10^9$ yr (ref. 2), $\lambda_p = 4.72 \times 10^{-10}$ yr $^{-1}$, $\lambda_e = 0.584 \times 10^{-10}$ yr $^{-1}$, $^{40}\text{K}/\text{K} = 0.0119$ atom %).

The samples were heated by a resistance heater in a gas extraction furnace for periods of 1 h at a sequence of temperatures up to 1,600° C. The argon evolved at each temperature step was cleaned by a heated Ti getter and analysed in a mass spectrometer. Atmospheric ^{40}Ar corrections were applied on the basis of furnace blanks measured before and after the introduction of samples to the furnace and are typically less than 1%. ^{39}Ar has been corrected for Ca derived ^{39}Ar (ref. 3). Both rocks contain cosmogenic and trapped (solar type) ^{38}Ar and ^{36}Ar . ^{40}Ar has been corrected for cosmogenic and trapped ^{40}Ar assuming $(^{40}\text{Ar}/^{36}\text{Ar}) = 1 \pm 1$ for both components. To calculate cosmic-ray exposure ages the proportion of cosmogenic ^{38}Ar has been calculated assuming $(^{36}\text{Ar}/^{38}\text{Ar}) = 5.25$ and 0.63 for trapped and cosmogenic components respectively.

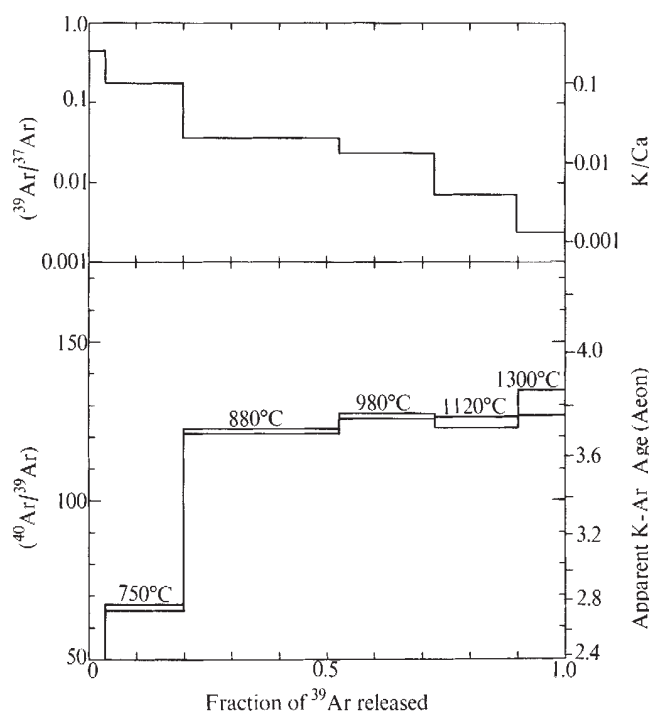


Fig. 1 Argon release pattern for Apollo 17 subfloor basalt 75055. The temperatures are extraction furnace temperatures. The 1,300° C fraction contains a large (2%) and uncertain correction for cosmogenic ^{40}Ar . $T = 3.75 \pm 0.05$ aeons.

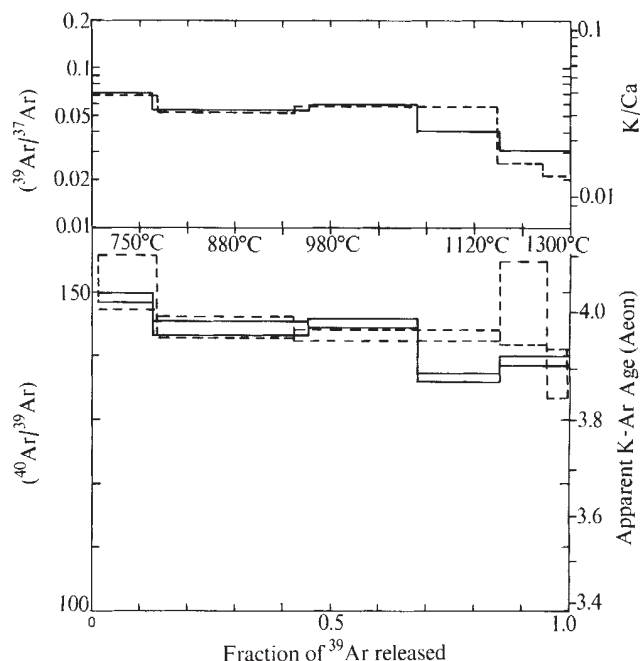


Fig. 2 Argon release pattern for Apollo 17 anorthositic gabbro 76055. The age quoted is based on the major release (880° C and 980° C) only. —, Matrix; ---, clast. $T = 3.98 \pm 0.05$ aeons.

In Figs. 1 and 2 we present the $(^{39}\text{Ar}/^{37}\text{Ar})$ ratio and the corrected $(^{40}\text{Ar}/^{39}\text{Ar})$ ratio plotted as a function of the proportion of ^{39}Ar released. We also show the (K/Ca) ratios and apparent K-Ar ages to which the argon isotope ratios correspond (see, for example, ref. 3).

The argon release pattern of 75055 is quite similar to patterns observed previously in lunar basalts^{4,5}. The low $(^{40}\text{Ar}/^{39}\text{Ar})$ ratio in the initial release indicates a small loss of ^{40}Ar (12%), probably as a result of solar heating, and the corresponding $(^{39}\text{Ar}/^{37}\text{Ar})$ ratio indicates that the loss is associated with potassium rich sites, probably an interstitial K-rich glass phase^{6,7}. On the basis of the high temperature release, an age of 3.75 ± 0.05 aeon is deduced for 75055.

Table 1 Representative ^{40}Ar - ^{39}Ar Ages of Lunar Samples

Sample type	Age (aeon)	Reference
Mare type basalts		
A11, high K	3.56 ± 0.09	4, 5
A11, low K	3.83 ± 0.09	4, 5
12002, 12051, 12065 (A12)	3.26 ± 0.06	5
15555 (A15)	3.31 ± 0.03	10
75055 (A17)	3.75 ± 0.05	This communication
Highland samples		
12013 (A12, "granitic" breccia)*	4.03 ± 0.07	5
14310, 14073 (A14, basalt)	3.88 ± 0.05	2, 7
14053 (A14, basalt)	3.95 ± 0.05	2
15382 (A15, KREEP basalt)	3.90 ± 0.05	†
15415 (A15, anorthosite)	4.05 ± 0.15	3
62295 (A16, norite)	3.87 ± 0.05	†
67075 (A16, anorthosite)	4.06 ± 0.05	†
L2015 (Luna 20, 8 mg, anorthosite fragment)	4.0 ± 0.3	†
76055 (A17, anorthositic gabbro)	3.98 ± 0.05	This communication

* The unique rock 12013 is listed with the highland samples somewhat arbitrarily on the basis of its age.

† D. T., T. H. C. and C. J. Y., presented at the 4th Annual Lunar Science Conference and submitted for publication in the *Proceedings* (1973).

The age of 75055 is comparable to the average age, 3.84 ± 0.09 aeon, determined previously for three Apollo 11 low-K basalts (Table 1), and confirms that the period of extrusion of

mare basalts on the Moon began as early as 3.8 aeon. The absence of intense cratering in the basalt flows at the Apollo 17 site places constraints on the early bombardment history of the lunar surface. For example the Imbrium event must have occurred before 3.75 aeon. We have argued elsewhere that this event occurred at 3.89 aeon ($\dagger$, legend to Table 1).

The argon release pattern of 76055 does not show effects of appreciable argon loss but does have some structure. The initial release of both matrix and clast shows a high ($^{40}\text{Ar}/^{39}\text{Ar}$) ratio whereas in the high temperature release from the matrix the ratio decreases. Similar effects have been observed in Apollo 14 breccias² and are not fully understood. The effects in 76055 are relatively small and over most of the release pattern the ($^{40}\text{Ar}/^{39}\text{Ar}$) ratio is not significantly different from the overall ratio. An age of (3.98 ± 0.05) aeon is calculated for both samples based on the major release at 880°C and 980°C . The ages based on the overall release are 3.96 aeon and 3.99 aeon for matrix and clast respectively. From a comparison of the ($^{39}\text{Ar}/^{37}\text{Ar}$) release patterns it is clear that the chemistry of both samples was very similar.

The age of 76055 is compared to the ages determined for highland material returned by earlier missions in Table 1. The concentration of ages around 4.0 aeon and the absence of ages in the period 4.0 to 4.6 aeon are the most obvious features of the distribution of ages which must be accounted for. Two classes of hypothesis may be put forward to explain the age pattern. The intense bombardment of the lunar surface in the period 4.0 to 4.6 aeon may have been responsible for heating and consequent argon loss from the highland samples. The predominance of clastic rocks and evidence of recrystallization in many of the highland rocks lend support to such hypotheses. The heating effects responsible may indeed be dominated by a small number of very large impacts in the regions visited specifically the Imbrium event and (for Apollo 17) the Serenitatis event. The location of the Apollo 17 site in the highlands surrounding the Sea of Serenity raises the possibility that the age of 76055 may indicate the time of the Serenitatis impact.

The possibility of explaining the age distribution in terms of the heating effects associated with capture of the Moon by the Earth cannot be ruled out for the period 4.6 to 4.0 aeon but the capture hypothesis and its associated tidal effects must face the additional constraint of the circularity of lunar craters⁸.

A second class of hypothesis regards the ages as true crystallization ages resulting from melting in the subcrustal regions as a result of internally generated heat. The ages of the mare basalts are usually accounted for as crystallization ages^{4,6}. Although such hypotheses may not be realistic as applied to the ages of clastic rocks it may still be possible to explain the anorthosite ages in this way³. The significance of the anorthosite ages is crucial to an understanding of early lunar history but at the present time is an open question. Both classes of hypothesis must satisfy the evidence from Rb-Sr measurements (such as rock 12013) for the early formation of a radioactive lunar crust 4.5 aeon ago⁹.

We have determined cosmic-ray exposure ages for 75055 and 76055 on the basis of the ratio of cosmogenic ^{38}Ar to ^{37}Ar produced artificially from Ca^{2+} . The corresponding ($^{38}\text{Ar}/\text{Ca}$) ratios are: $(1.38 \pm 0.06) \times 10^{-6}$ ml. (STP) g^{-1} for 75055 and $(1.91 \pm 0.10) \times 10^{-6}$ ml. (STP) g^{-1} for both clast and matrix of 76055. The corresponding exposure ages, based on a nominal ^{38}Ar production rate of 1.4×10^{-8} ml. (STP) g^{-1} m.y.⁻¹ (ref. 2), are: 98 ± 5 m.y. (75055) and 136 ± 7 m.y. (76055).

The exposure ages are a measure of the integrated irradiation history of the samples. 76055 was a small rock lying on the mountain slope at station 6 and has no clear relationship to any cratering event in that vicinity. We can infer no immediate conclusions from our measurement of its exposure age but must await a more statistically significant number of measurements on material from station 6. By contrast 75055 seems to be clearly associated with the impact which produced Camelot crater and we therefore infer an age of (98 ± 5) m.y. for the age

of Camelot. A number of crater ages have now been determined on the Apollo missions (Apollo 14 Cone crater, 26 m.y. (ref. 2); Apollo 16 North Ray, 46 m.y. ($\dagger$, see legend to Table 1); Apollo 17 Camelot, 98 m.y.) and provide a fairly well defined absolute time scale with which to compare relative ages based on crater morphology.

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Note added in proof. Since the paper was written a duplicate analysis of 75055 has yielded results identical to those presented here.

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- ¹ Merrihue, C. M., and Turner, G., *J. Geophys. Res.*, **71**, 2852 (1966).
- ² Turner, G., Huneke, J. C., Podosek, F. A., and Wasserburg, G. J., *Earth Planet. Sci. Lett.*, **12**, 19 (1971).
- ³ Turner, G., *Earth Planet. Sci. Lett.*, **14**, 169 (1972).
- ⁴ Turner, G., *Geochim. Cosmochim. Acta*, Suppl. 1, **2**, 1665 (1970).
- ⁵ Turner, G., *Earth Planet. Sci. Lett.*, **11**, 169 (1971).
- ⁶ Lunatic Asylum, *Science*, **167**, 463 (1970).
- ⁷ Turner, G., Huneke, J. C., Podosek, F. A., and Wasserburg, G. J., *Geochim. Cosmochim. Acta*, Suppl. 3, **2**, 1589 (1972).
- ⁸ Öpik, E. J., *Astron. J.*, **66**, 60 (1971).
- ⁹ Lunatic Asylum, *Earth Planet. Sci. Lett.*, **9**, 137 (1970).
- ¹⁰ Podosek, F. A., Huneke, J. C., and Wasserburg, G. J., *Science*, **175**, 423 (1972).

Orange Soil from the Moon

THE discovery of an orange-coloured soil on the Moon by the Apollo 17 astronauts last December led to much excitement and speculation. Dr Jack Schmitt, the geologist on the mission, realized that such yellow to orange coloration in rocks on Earth is usually due to hydrated iron oxides. In a volcanic region, these minerals are produced from water-rich vapours reacting with the lavas and emanating from gas-vents or "fumaroles" associated with the volcano.

Before this discovery, all the evidence from lunar volcanic rocks pointed to the absence of water (except possibly as a transient phase quickly lost from the lunar environment due to the low gravitational field). The rarely discovered hydrous minerals (such as goethite) were confined to a few of the rocks fragmented by meteoritic impact (breccias), and there is additional evidence that certain classes of external meteorite, such as carbonaceous chondrites, as well as comets, could contribute water as well as other volatile constituents to the lunar soils and breccias. Dr Schmitt was on the alert for evidence of recent volcanism in the Taurus-Littrow region, in view of some morphological evidence in support of that possibility. His conclusion while on the Moon was that Shorty crater could be associated with young volcanic activity and that the orange coloration in the adjacent soil was perhaps due to fumarolic activity. Scientists on Earth concluded that, if this were so, oxidation of the iron-minerals in the lava debris implied the presence of water in the lunar interior, perhaps up to recent times, which was released to the surface during volcanic eruptions. If so, and the water remained long enough to react with the surface lavas, then the role of water during earlier processes on the Moon would be more significant than had been envisaged.

We were allocated a small sample of orange soil (0.25 g) together with a basaltic lava specimen which reached Durham on February 24, and we presented our data and conclusions at the Fourth Lunar Science Conference in Houston (March 8). In this communication we show how it was possible to reach a precise conclusion from chemical analysis, and to clarify the situation regarding the orange soil. We conclude that the material was not produced by volcanic processes.

The soil sample (74220,60) consists almost entirely of tiny glassy spheres or beads (average diameter 0.1–0.2 mm); they range from pale yellow to black in colour, but are predominantly orange. Less than 5% of the sample consists of mineral fragments, chiefly plagioclase feldspar. Electron probe analysis of spheres of varying colour gave the compositions in Table 1. The orange colour is almost certainly due to the high titanium content, as similar orange glasses from other missions show high TiO_2 values. The content of TiO_2 is not, however, the most remarkable feature of these glasses, as some of the mare basalts from Apollo 11 and Apollo 17 sites show higher contents. The unusual feature is the low aluminium, giving Al_2O_3 values much lower than in basaltic lavas. Calculation of the normative mineral contents of the glasses, for example (Table 1), shows only 14.3% to 17.4% normative plagioclase feldspar. Conversely, the normative contents of mafic minerals are very high, with up to 43% normative olivine in the black glassy spheres. This unusual composition can best be explained as due to the melting of mineral debris on the lunar surface by meteorite impact, giving splashes of liquid droplets. A similar origin has been advocated for glassy beads from previous mission collections, where the beads include feldspathic (colourless) and pyroxenitic (green) compositions.

Table 1 Apollo 17 Orange Glass (74220,60) with Comparisons

	Typical orange*	Range	Black glass †	Apollo 11 orange glass	Mare 4 basaltic glasses
SiO_2	38.98	37.8–39.0	38.06	42.21	37.64
TiO_2	9.23	8.9–9.6	7.26	8.47	12.04
Al_2O_3	5.67	5.6–5.7	4.77	11.32	8.46
FeO	22.44	22.2–22.8	22.57	16.87	19.93
MnO	0.24	0.24–0.28	0.28	0.20	ND
MgO	14.47	13.8–15.1	20.40	8.67	10.49
CaO	7.31	7.1–7.5	5.57	10.86	8.81
Na_2O	0.43	0.34–0.43	0.30	0.28	0.54
K_2O	0.04	0.04–0.04	0.04	0.21	0.13
Cr_2O_3	0.66	0.63–0.72	0.66	0.32	0.48
Total	99.47		99.91	99.41	98.52

* Typical of yellow, orange, red-brown and some black glasses.

† Example of some black glasses that are heterogeneous and variable.

Typical orange norm: Or 0.24, Ab 3.66, An 13.49, Di 18.79, Hy 24.10, Ol 21.12, Cm 0.97, Ilm 17.62.

Norm minerals (black): Plag 14.3 (An81), Cpx 13.1, Opx 14.9, Ol 42.9 (Fo69), Opaques 14.8.

Norm minerals (orange): Plag 17.4 (An78), Cpx 18.8, Opx 24.1, Ol 21.1 (Fo65), Opaques 18.6.

Approximate relations: 100 parts orange + 38 parts olivine = black.

The glassy spheres are generally free from residual crystals that could be attributed to the impacting body (no nickel-iron particles, for example). They do, rarely, contain fragments of ilmenite (suggesting residual grains from the impacted source rocks) and minute feathery crystals of ilmenite (orange glasses) or olivine (black glasses) suggestive of the melt being oversaturated in these components, and hence they were expelled first during devitrification.

An X-ray fluorescence analysis of the bulk soil sample was then undertaken, using a non-destructive method. The results (Table 2) are surprising in that the contents of zinc, copper and nickel are extremely high. This is particularly strong, further evidence against the notion that the glass beads could be due to volcanic eruption of basaltic liquid in the form of fountain droplets. Trace element data are not yet available for the Apollo

17 basalts, but their mineralogy and major-element chemistry indicate very close similarities to the Apollo 11 titaniferous mare basalts. A comparison (Table 2) shows that such basalts have extremely low contents of Zn, Cu and Ni (an Apollo 17 basalt contains Ni-free iron also). In fact, the "orange soil" is even richer in Zn and Cu than the Apollo 11 soil which contains meteoritic debris as well as local basalt fragments.

Table 2 Orange Glass Sample (74220,60). Semi-quantitative, Non-briquetted (0.25 g) XRF Analysis (p.p.m.)

	Orange sample	Apollo 11 basalt (10045)	Apollo 11 soil (10085)
Ba	88	44	257
Nb	10	12	15
Zr	170	219	351
Y	45	85	124
Sr	179	145	159
Rb	<1	1	3
Zn	262	3	19
Cu	42	6	16
Ni	65	4	117

We conclude, therefore, that the impacting material was unusually rich in Zn and Cu, and that it has been dissolved in the liquid produced by the impact event. Evidence presented at the recent Houston conference by other investigators indicates that cometary impact may be responsible for high levels of zinc and chlorine in Apollo 16 rocks, and therefore the unusual abundance of glass beads at the Apollo 17 site may be due to this type of impact. Although our evidence is aimed at distinguishing between impact and volcanic processes, other investigators reported data relevant to the two other problems raised by Dr Schmitt's observations. Analysis by Mössbauer spectroscopy showed the absence of ferric iron in the orange glasses (and hence a low oxidation state), and isotopic measurements showed the materials not to have been formed by recent events on the Moon. Hence we have no evidence for young, hydrous volcanism on the Moon and the orange and black soil seems to be a local, strong concentration of impact-generated melts.

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Lunar Tides and Magnetism

RECENT observations and studies of the lunar surface based on Apollo and Explorer flights¹ show that some rocks brought to Earth are magnetized, that there are on the Moon weak, local, fairly randomly oriented magnetic fields but no overall poloidal field, that the magnetic perturbation of the solar wind by the surface of the Moon as observed by lunar orbiters is concentrated at latitudes lower than about 40° (ref. 2) and, finally, that the chemistry of the surface suggests high present and past radioactivity³. Here I propose a model, based on these observations, which may lead to the understanding of the origin of lunar magnetism a few billion years ago.

It has been pointed out¹ that the observed lunar magnetism cannot be accounted for either by terrestrial or by solar magnetic fields. The possibility that the Moon once had a sufficiently hot liquid core in which a turbulent convective motion could have produced a magnetic dynamo driven either by a thermal gradient^{4,5} or by precession⁶ seems to be marginal for energetic or electromagnetic reasons⁷. The existence of a

liquid core is also in seeming conflict with the dynamic stability of the Moon⁸. It is interesting, therefore, to inquire whether a magnetic field could have been generated in an outer liquid shell. The studies of Gast⁹ and Wood *et al.*³ of the lunar composition and of its present and past radioactivity lead to the conclusion that at an early epoch, some 3 to 4 × 10⁹ yr ago, there existed a thin anorthosite crust (density 2.9 g cm⁻³) which was floating on a few hundred kilometres thick layer of molten gabbro (density 3 to 3.3 g cm⁻³) which, in turn, was supported by solid olivine (density 3.2 to 4.4 g cm⁻³). This transient situation was caused presumably by a relatively brief pulse of heat generated by various short-lived radioactive elements, primarily ²⁶Al, which tended to concentrate in the liquid fractions.

If such an external liquid shell indeed existed then, in principle, a magnetic field could have been generated in it either by thermally driven convection in analogy to what has been suggested for Jupiter^{10,11}, or by tidal motions produced by Earth⁷. In order to assess the validity of these two mechanisms it is necessary to estimate first the average velocities v produced in the liquid. Using Fish *et al.*'s¹² numerical values for ²⁶Al and for its initial concentration in a 100 km thick layer, the layer must have become liquid in a few million years and at that time the outward heat flux Q was of the order 10⁴ erg s⁻¹ cm⁻² implying a surface temperature of about 100 K if no other heat source was present. The simple mixing length theory of thermally driven convection¹³ with the appropriate numerical quantities leads to the expression

$$v^3 = 8 \times 10^{-9} / Q \text{ cm}^3 \text{ s}^{-3}$$

With a mixing length l of the order of 1 to 100 km convective velocities are 2 to 10 cm s⁻¹. Radioisotopes other than ²⁶Al would lead¹² to much later melting, lower heat fluxes and lower convective velocities.

For evaluation of the tidal mechanism I assume that the outside crust is so thin that it is easily deformable and does not affect the motions in the liquid. The perturbing potential relative to the Moon produced by Earth in the liquid shell at a point P on the lunar surface¹⁴ is

$$\Omega = (3/2) \gamma E r^2 D^{-3} (1/3 - \cos^2 \theta) \quad (1)$$

where γ is gravitational constant, E is mass of the Earth, r is radius of the Moon, D is distance between Earth and Moon and θ is the angle between the line joining the centres of the two bodies and the lunar radius leading to point P (or approximately the lunar latitude). The average value of the perturbing force field (radial and tangential)

$$\overline{\nabla \Omega} \sim (3/2) \gamma E r D^{-3} \quad (2)$$

is about 2 × 10⁻³ cm s⁻², some 23 times larger than the corresponding lunar gravitational field responsible for tides on Earth. It follows that the solar influence, which on Earth is comparable to the lunar influence, is negligible for the Moon. The problem of evaluating velocities v in tidal motions even in a liquid layer of uniform thickness is a formidable one. But an approximate estimate of an average velocity v can be made applying to a spherical shell Malkus's⁶ assumption that the perturbing force is of the same order of magnitude as the Coriolis force per unit mass $[2 \omega \times v]$ where ω is angular velocity. If at the early epoch the rotation of the Earth and the Moon had not yet been slowed, both could have had a period of less than 10 h, typical of initial periods of most bodies in the Solar System¹⁵⁻¹⁷. On the other hand, the very low dynamic symmetry of the Moon may have existed at that time and may already have affected its rotation so that longer periods should be also considered. For periods between 10 h and 30 day the corresponding velocities are 6 to 400 cm s⁻¹.

A self-sustaining magnetic dynamo can exist¹⁸ if the magnetic Reynolds number $G = \mu L \sigma v$ is greater than 10. Here μ is permeability, L is linear dimension and σ is electrical conductivity. Putting $\mu \sim 1$ and L a few hundred kilometres gives $1 < G < 50 (\Omega \text{ cm})^{-1}$ which, using Dyal and Parkin's data for basalt¹⁸, indicates that the temperature of the liquid gabbro had to be not less than 1,900 K for the convective mechanism and not less than 1,600 K for the tidal mechanism. For the Apollo 11 sample¹⁹ the corresponding minimum temperatures are 1,750 and 1,300 K. Thus the tidal mechanism seems to pose less stringent requirements for the generation of a magnetic field in the liquid shell than thermal convection. On the other hand, a more detailed quantitative evaluation of the history of the transient melting might alter this conclusion.

The strength of the magnetic field H produced in this way depends, of course, upon the actual configuration and topology of the currents and is very difficult to estimate. Recent studies²⁰ indicate that the generation of magnetic fields in rotating bodies is closely related to the presence of non-reflexion-symmetric turbulence such as one would expect to exist in tidal motions. By comparing the Coriolis and perturbing forces with the Lorentz force per unit mass $[(4\pi\rho)^{-1} (\nabla \times H) \times H]$ where ρ is the density of the liquid⁶ one obtains, for currents with an effective radius of curvature of the order of kilometres, local fields of 10 gauss or more, which is very high. Clearly, for realistic and complicated boundary conditions, somewhat resembling those for tides in Earth's oceans, the local velocities and the local magnetic fields could be appreciably lower.

This admittedly very simplified model leads to the conclusion that the outer layer of the Moon could have been magnetized by local, more or less randomly oriented, magnetic fields produced by tidal currents, that there would be no overall poloidal field and that the magnetization would be observable primarily near the lunar equator where, from equation (1), the tide producing force has its maximum. All these conclusions seem to be in accord with the observations mentioned earlier and also the conflict with the stability requirements of the Moon is avoided. A detailed quantitative analysis of the tidal motions for a less idealized distribution of the liquid phase should be made. Such a distribution could be perhaps deduced from the most recent surveys of chemistry and morphology of lunar rocks and from the configuration of the surface magnetization and of surface fields.

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- ¹ Sonett, C. P., and Runcorn, S. K., *Comments on Astrophys. and Space Phys.*, **3**, 49 (1971).
- ² Sonett, C. P., and Mihalov, J. D., *J. Geophys. Res.*, **77**, 588 (1971).
- ³ Wood, J. A., Dickey, J. S., jun., Marvin, U. B., and Powell, B. N., *Proc. Apollo 11 Lunar Sci. Conf.*, **1**, 965 (1972).
- ⁴ Elsasser, W. M., *Phys. Rev.*, **55**, 489 (1939).
- ⁵ Bullard, E. C., in *The Earth as a Planet* (edit. by Kuiper, G. P.) (University of Chicago Press, Chicago, 1954).
- ⁶ Malkus, W. V. R., *Science*, **160**, 259 (1968).
- ⁷ Smoluchowski, R., *The Moon*, **6**, 48 (1973).
- ⁸ Lunar Science Institute, *Post-Apollo Lunar Science*, Lunar Science Institute, Houston, Texas (1972).
- ⁹ Gast, P. W., *The Moon*, **5**, 121 (1972).
- ¹⁰ Hide, R., *Magnetism and Cosmos*, 378 (Oliver and Boyd, Edinburgh, 1967).
- ¹¹ Smoluchowski, R., *Phys. Earth Planet. Inter.*, **6** (1972).
- ¹² Fish, R. A., Goles, G. G., and Anders, E., *Astrophys. J.*, **132**, 243 (1960).
- ¹³ Schwarzschild, M., *Structure and Evolution of the Stars* (Princeton University Press, Princeton, 1958).
- ¹⁴ Lamb, H., *Hydrodynamics* (Cambridge University Press, 1932).
- ¹⁵ Alfvén, H., *Icarus*, **3**, 57 (1964).
- ¹⁶ Giulì, R. T., *Icarus*, **8**, 301 (1968).
- ¹⁷ Mitra, V., *Astron. Astrophys.*, **4**, 263 (1970).
- ¹⁸ Dyal, P., and Parkin, C. W., *J. Geophys. Res.*, **76**, 5947 (1971).
- ¹⁹ Nagata, T., Rikitake, T., and Kono, M., *Space Res.*, **9**, 85 (1971).
- ²⁰ Krause, F., Rädler, K. H., and Steenbeck, M., *The Turbulent Dynamo*, Nat. Center Atmos. Res., Tech. Note 1A-60 (1971).

Rate of Horizontal Fault Displacement in New Zealand

WELLMAN¹ has estimated horizontal faulting rates in New Zealand. His presentation, however, takes little heed of published data relevant to recent crustal movements in New Zealand and his inferences are extremely tenuous.

Wellman states: "From seafloor spreading . . . the rate [of horizontal displacement] is estimated to be about 34 mm per year" and quoted Christoffel² and Wellman³ in support. Christoffel, however, estimated "relative average rate of movement between 4.2 and 5.8 cm a year". The estimate of 34 mm per year is Wellman's alone.

In estimating an age of 10,000 yr for the Waiohine and other aggradation surfaces Wellman ignored the radiocarbon dates from which the dating of correlative aggradation surfaces in the South Island has been developed. These dates^{4,5} indicate an age of about 18,000 yr for the latest principal aggradational surfaces developed during the latest (Otira) glaciation.

Wellman stated that at Turakirae Head "the highest [beach] ridge seems to be roughly 6,300 yr old. Radiocarbon dating of samples from beds a few metres below the highest beach ridge supports this estimate". In fact the radiocarbon dates referred to⁶ are from a different section on a different structure 70 km away across the regional strike of several important structures, and the dating of the beach ridge depends on complex relations of tectonism and sea level rise⁷.

We are unaware of the evidence on which Wellman bases the active dextral fault shown offshore off the southeast of the North Island; this was first postulated in 1971 by Wellman⁸ without any substantiation. Later³ in 1971 he merely noted that it was "reasonably well-defined by bathymetry to the northeast".

Wellman's assumption of a constant average rate of vertical movement ignores the evidence⁹ of decreasing rate and reversals of vertical movements at many active faults since the formation of the last main aggradational surface. This makes calculations based on rates of vertical movement suspect. Wellman has to assume a changing average rate of horizontal movement at Waiohine rather than the constant rate for transcurrent faults in general that he has accepted previously^{10,11}.

The Cape Turakirae beaches lie on the anticlinal fold between the Wellington and Wairarapa Faults. Uplift there will result whenever accumulated strain along the fold is relieved by triggering from earthquakes associated with movement at any of the major faults in the region, not only at the Wairarapa fault, which displaces the Waiohine terraces. Uplift at Turakirae may thus be significantly more frequent than movement on the Wairarapa Fault at the Waiohine terraces. Wellman's inference that the same earthquakes caused movement in both places is not valid.

Wellman's ratio will remain the same if a constant factor exists relating frequency of uplift at Turakirae to faulting at Waiohine. No inference of age of the Waiohine surface and by extension of the rate of horizontal movement is possible without knowledge of such a factor. The Waiohine surface could well be several times older than Wellman assumes.

If one were to accept Wellman's 240 m summation of post-aggradation movement at the major faults, and an age of 18,000 yr for the latest principal aggradation surface, the rate of strain would be 13 mm yr⁻¹. This is little more than one-third of Wellman's estimate of 34 mm yr⁻¹ and an even smaller part of either of Christoffel's estimates, all from seafloor spreading. Conversely, for Wellman's rate of 34 mm yr⁻¹ the principal aggradation surfaces must be 7,000 yr old, which is unrealistic.

Suggate¹² has maintained and Freund¹³ has accepted that there is no good evidence of large horizontal displacements in post-glacial time along the central section of the Alpine Fault, where Wellman's reconstruction would require the rate of strain (34 mm yr⁻¹) to be the sum of the rates on the faults of the shear belt to the northeast. Freund¹³ has put forward

one possible hypothesis whereby the horizontal movement on these faults need not be transmitted to the Alpine Fault.

We urge those dependent on published literature for their knowledge of Recent crustal movements in New Zealand to study that literature as fully as possible. New Zealand's key position in relation to floor spreading makes such a study necessary.

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¹ Wellman, H. W., *Nature*, **237**, 275 (1972).

² Christoffel, D. A., *Roy. Soc. NZ Bull.*, **9**, 25 (1971).

³ Wellman, H. W., *Pan Pacific Meeting on Geological Structure of Pacific Area* (Univ. Western Australia, in the press, 1971).

⁴ Suggate, R. P., *NZ Geol. Surv. Bull.*, n.s., **77** (1965).

⁵ Suggate, R. P., and Moar, N. T., *NZ J. Geol. Geophys.*, **13**, 742 (1970).

⁶ Singh, L. J., *Roy. Soc. NZ Bull.*, **9**, 217 (1971).

⁷ Wellman, H. W., *J. Geoscience, Osaka City Univ.*, **10**, 123 (1967).

⁸ Wellman, H. W., *Roy. Soc. NZ Bull.*, **9**, 211 (1971).

⁹ Lensen, G. J., *Bull. NZ Soc. Earthq. Eng.*, **3**, 131 (1970).

¹⁰ Wellman, H. W., *Tectonophysics*, **12**, 199 (1971).

¹¹ Wellman, H. W., *Geologischen Rundschau*, **43**, 48 (1955).

¹² Suggate, R. P., *Trans. Roy. Soc. NZ, Geology*, **2**, 105 (1963).

¹³ Freund, R., *NZ Geol. Surv. Bull.*, n.s., **86** (1971).

Magnetism and Archaeology

A RECENT discussion about reversed geomagnetic events in the Brunhes epoch contains the statement that no archaeological materials are known to be reversely magnetized¹. This may be true for the specific region that is considered in the article, but it is not true in general. G. Folgheraiter in "Rendi Conti dei Licei", 1896, 1899; *Archives des sciences physiques et naturelles* (Geneva), 1899; *Journal de physique*, 1899; and P. L. Mercanton, in "La methode de Folgheraiter et son role en geophysique", *Archives des sciences physiques et naturelles*, 1907, reported observations made on clay fired in kilns by the Etruscans and Greeks. Their results indicate that in the eighth century BC the Earth's magnetic field was reversed.

In 1896 Giuseppe Folgheraiter made studies of Attic (Greek) and Etruscan vases of various centuries, starting with the eighth century BC. The observations were made on clay fired in kilns. The position of the ancient vases during firing is known. They were fired in a standing position, as indicated by the flow of the glaze. The magnetic inclination or the magnetic dip of the iron particles in the fired clay indicates the nearest pole during time of firing. His conclusion was that in the eighth century BC the Earth's magnetic field was reversed at least in Italy and Greece.

P. L. Mercanton of Geneva, studying the pots of the Hallstatt age from Bavaria (about 1000 BC) and from the Bronze Age caves in the region of Lake Neuchâtel, came to the conclusion that about the tenth century BC the direction of the magnetic field differed only slightly from its present direction. His material was of an earlier date than that used by Folgheraiter but, checking on the method and results of Folgheraiter, Mercanton found them correct².

This work has been brought to the attention of Elizabeth K. Ralph, Associate Director, the University of Pennsylvania Museum, who is presently investigating magnetic field reversals. She is considering investigation of material taken from kilns dating around the eighth century BC. These kilns were recently unearthed in Sarepta by James B. Pritchard who is also asso-

ciated with the museum. This work could provide additional valuable data in the investigation of magnetic field reversals.

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¹ *Nature*, **239**, 305 (1972).

² Velikovsky, I., *Earth in Upheaval*, 146 (Doubleday, 1955).

BIOLOGICAL SCIENCES

Metabolic Availability of Vitamin C in the Guinea-pig

THE results of studies in which L-gulonolactone-1-¹⁴C, the precursor of L-ascorbic acid in the rat, was administered to guinea-pigs showed that the guinea-pig is apparently unable to synthesize vitamin C¹⁻³. This has been attributed to the absence of gulonolactone oxidase from the liver microsomes of the guinea-pig; because this enzyme is the last one in the series for converting glucose to ascorbic acid it completely blocks endogenous synthesis. Synthesis of ascorbic acid has been demonstrated in rats: gulonolactone oxidase is present in their livers⁴⁻⁶. A defect, probably a conditional lethal mutation⁷, in the gene controlling the synthesis of this enzyme in guinea-pigs produces an inactive enzyme or its complete absence. They are therefore dependent on exogenous vitamin C. This genetic disease has been named hypoascorbemia because of the low levels of ascorbic acid in the blood which are pathognomonic of the condition⁸. Neither the sex nor the age of the animals in these experiments was stated, and there was no indication of the state of ascorbic acid tissue saturation or of the scorbutic condition of the guinea-pigs.

Diet, age and sex greatly influence the metabolism of ascorbic acid in rats⁹⁻¹². In spite of the evidence for the uniform presence of a conditional lethal genetic defect in guinea-pigs, Williams and Deason¹³ demonstrated great variability in guinea-pig requirements for exogenous vitamin C in order to prevent the development of scurvy. Guinea-pigs do not develop scurvy on account of an excessively high rate of destruction of L-ascorbic acid¹⁴, so it seems that some guinea-pigs have greater ability to synthesize ascorbic acid than others. The evidence of Burns¹⁵ suggests that these guinea-pigs must have higher concentrations of gulonolactone oxidase in their livers, because their genetic defect is not as dominant as in the other less well-endowed members of the species¹⁶. Stone¹⁶ has suggested that the extent of this defect could be determined by observing the development of scorbutic symptoms and measuring the ability of the guinea-pig to synthesize ascorbic acid while on a diet deficient in ascorbic acid. The results of such an investigation in the guinea-pig are described here.

Duncan-Hartley guinea-pigs, initially weighing 380-400 g, were used. They were housed singly and maintained on a normal diet of rabbit pellets containing 27 mg ascorbic acid/100 g pellets, and free access to water, of which each animal drank about 30 ml. daily. After 2 weeks of acclimatization to the diet and checking to exclude individual pathology, they were transferred to the scorbutogenic diet as described by Hughes and Hurley¹⁷; this contained ground oats, wheat bran, and skimmed milk, modified only by the addition of 0.5% instead of 1.0% supplementary vitamins and salts, excluding ascorbic acid. No vitamin C could be detected in this diet by chemical analysis. On transfer to the diet, supplementary ascorbic acid (50 mg 100 ml.⁻¹) was added to the drinking water so that each animal received about 15 mg supplementary vitamin C daily. This treatment was maintained for a further 2

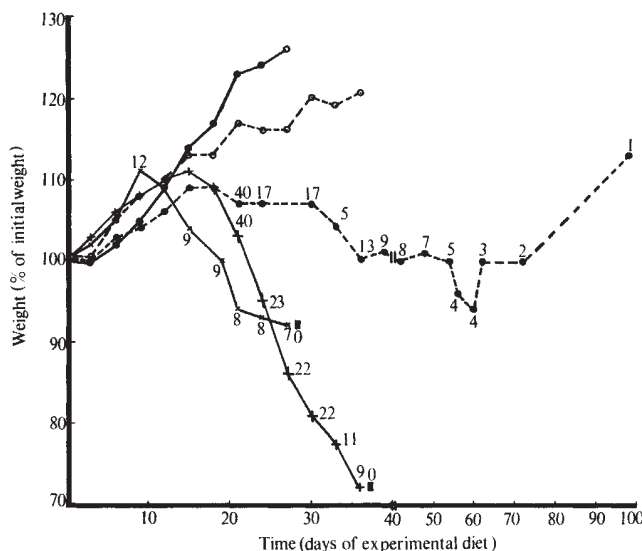


Fig. 1 Effect of ascorbic acid supplementation and deficiency on guinea-pig growth and survival. Survival times and alteration in weight of guinea-pigs maintained on a diet free of ascorbic acid. ○---○, Control females, diet+15 mg ascorbic acid daily intraperitoneally. ○—○, Males and females, diet+ supplementary ascorbic acid 100 mg daily by stomach tube. ×—×, Male guinea-pigs, diet alone. ●...●, Female guinea-pigs, Survivors, diet alone. +—+, Female guinea-pigs, Diers, diet alone. ■, 100% fatality. The numbers of guinea-pigs at each point of time in the males on diet alone, the female Survivors and the female Diers, are indicated.

weeks after which animals failing to maintain a steady growth pattern or showing impaired health were discarded. Following this 4 week maintenance period, a control group of females continued treatment with the scorbutic diet and 15 mg of supplementary ascorbic acid was given daily by intraperitoneal injection; a supplemented group, comprising equal numbers of males and females, received the diet together with 100 mg ascorbic acid given daily to individual animals by stomach tube; and separate groups of male and female guinea-pigs continued to receive the diet without any supplementary vitamin C (scorbutic groups). The animals were weighed every 3 days at 1000 h to avoid variations introduced by circadian rhythms. They were divided randomly into groups of six animals so that the killing order for tissue analysis was predetermined during the early part of the experiment. Guinea-pigs were killed at 1200 h by exsanguination after stunning. Ascorbic acid was measured, after extraction in 10% metaphosphoric-acetic acid mixture, by the phenyl hydrazine method¹⁸⁻²⁰. Gulonolactone oxidase activity was detected in liver by a specific histochemical procedure using nitro-blue tetrazolium (Nitro BT), phenazine methosulphate, and potassium cyanide in the presence of L-1,4-gulonolactone²¹.

In Fig. 1 the changes in body weight of male and female guinea-pigs on the scorbutogenic diet are compared with the weight changes in the control group of female guinea-pigs receiving the scorbutogenic diet and 15 mg of vitamin C daily by intraperitoneal injection, and with the gain in body weight of the supplemented group receiving the diet with 100 mg ascorbic acid administered daily by stomach tube.

The control group gained weight steadily during the 36 days of the experiment. The supplemented group began to gain weight more rapidly than the control group after day 18. By day 24 the supplemented group was significantly heavier than the control group. Two separate experiments were carried out on male scorbutic groups. In the first experiment, they became slightly heavier than the control or supplemented groups during the first 12 days of the experiment, then quickly lost weight. They had lost all their additional weight by day 24. On day 27

their weight was less than at the beginning of the experiment, they showed scorbutic signs and were dying spontaneously. In the second experiment, two out of six animals were dead, two were moribund, and two were showing severe scorbutic signs and died on day 30. The mean liver and plasma ascorbic acid concentrations of the latter four animals were 1.06 ± 0.21 mg per 100 g and 0.055 ± 0.001 mg per 100 ml. respectively. Two separate experiments were also performed on the female scorbutic groups. Fifty-four animals started the scorbutogenic diet in the first experiment. Their record of survival has already been described²². Thirty-six received the diet in the second experiment and these animals either died, were deliberately killed as required for tissue analysis, or were subjected to special tests during the experiment. On day 72 two were still alive and gaining weight. The final survivor died on day 154 from infection following a series of vaginal examinations. The results are summarized in Fig. 1 and Table 1.

Table 1 Mean Ascorbic Acid Concentrations in the Livers (mg per 100 g) and Plasma (mg per 100 ml.) of Guinea-pigs Maintained on an Ascorbic Acid Free Diet

Day of diet	No.	Males Liver	Males Plasma	Female Survivors No.	Female Survivors Liver	Female Survivors Plasma	Female Diers No.	Female Diers Liver	Female Diers Plasma
0	6	27.48	0.43	6	20.42	0.88			
6	6	24.35	0.50	6	16.35	1.03			
12	6	16.08	0.37	6	7.17	1.02			
18	6	6.29	0.21	6	2.25	0.46			
24				3	2.08	0.25			
27	2	0.30	0.09						
30	4	1.06	0.06				11	2.16	0.12
32				2	3.90	0.17			
35				1	4.27	0.20			
36				3	5.64	0.22	6	2.97	0.08
62				1	4.69	0.57			
72				1	8.17	0.18			
98				1	11.04	1.40			

On day 24, the females were differentiated into potential Diers and potential Survivors on the basis of their growth patterns.

During the first 15 days, the scorbutic females gained weight almost as rapidly as the control and supplemented groups. By day 18 the scorbutic group had begun to lose weight. On day 24 they were significantly lighter than the female control group as has also been reported by Hodges and Hotston²³. The guinea-pigs in these investigations, however, did not receive ascorbic acid supplementation before the experiment, thus accounting for the appearance of a moribund condition by day 24. At this time scorbutic females could be divided into two groups, the potential Diers and the potential Survivors, on the basis of their growth patterns. From day 24 until the end of the experiment, the Diers showed a rapid and steady decrease in weight. On day 24 they were showing scorbutic symptoms. They all died during the first 36 days of the diet. The Survivors ceased to gain weight after day 18. They showed a gradual stepwise decline in weight until day 36 when their weight had fallen to the level at the beginning of the experiment. They maintained this initial weight, apart from a temporary relapse on day 60, until day 72 of the scorbutic diet after which the surviving guinea-pigs gained weight steadily and uniformly. Retrospective analysis of the weights of the Diers and Survivors before day 24 demonstrated that the Diers initially gained weight more rapidly than the Survivors. After day 24, the Diers lost weight much more rapidly than the Survivors. When any of the surviving female guinea-pigs died, they did not show severe scorbutic signs like those of the males or female Diers.

Food intake of males and females rose until day 12. On day 24 it had returned to its original level in the females, but it continued to fall in the males and was 57% of normal when they were dying. From day 27 until day 72 it remained at 70% of the original level in the surviving females. At the end of the

experiment the surviving females were consuming 82% of the initial group food intake.

Plasma and liver ascorbic acid concentrations were measured in males and in female Survivors and Diers as the experiment proceeded (Table 1). Those killed among the Diers were generally moribund at the time of death. Animals selected for tissue analysis among the Survivors had the lowest body weights. The numbers on each occasion were determined by the total number available in the group. Liver ascorbic acid values diminished in both sexes during the first month. The rate of fall was greater in the females. Liver ascorbic acid reached its minimum on day 24 when it was 10.6% of the initial value. The maximal rate of fall was later in the males, and took place when the decrease in body weight was most pronounced. On day 27 the liver ascorbic acid was 1% of the initial value in the males. After day 24 the mean liver ascorbic acid concentrations ceased to fall in the female Survivors. Ascorbic acid was present in relatively high concentration in the livers of the Survivors killed on days 72 and 98. On day 98 the liver ascorbic acid value was 54% of that in the normal animals killed before the diet was started.

Plasma ascorbic acid levels were elevated in both sexes at the beginning of the scorbutogenic diet but the elevation was maintained for 6 days longer in the females. They had reached a minimum in both males and females on day 30. The mean ascorbic acid concentration had begun to rise in the female survivors on day 32. Plasma levels were still diminishing in the Diers even though liver concentration had begun to rise slightly. When the diet was started, liver values began to fall while plasma levels were elevated. Subsequently the rise in plasma ascorbic acid only occurred after raised levels had been detected in the liver.

Table 2 Mean Weights of Surviving Guinea-pigs Maintained on Scorbutogenic Diet Alone, and the Effect of PCMB, 0.3 mg IMI on their Weights while Maintained on the Diet Alone or on the Diet Supplemented with Ascorbic Acid (15 mg Daily)

Type of treatment		No. of guinea-pigs	Days			
			0	1	4	8
Diet	PCMB		Mean weights (g)			
Scorb.	None	3	435	462	472	478
Scorb. + Suppl.	Daily	2	515	526	534	543
Scorb.	Daily	2	444	482	466	355

Day 1 corresponds to day 54 of the scorbutic diet and to day 4 of the supplemented diet.

Raised ascorbic acid concentrations in the liver before any change in plasma levels suggested that ascorbic acid originated in the liver. Staining of the liver of male and female rats with Nitro BT demonstrated the presence of gulonolactone oxidase. Similar and simultaneous treatment of the liver of the female guinea-pig maintained on the scorbutogenic diet for 98 days demonstrated the presence of brown granules within the hepatic centrilobular cells. The brown granules in the guinea-pig liver were identical in distribution and appearance to those demonstrated in rat liver after staining with Nitro BT. Similar brown granules were not visible in the liver of a male guinea-pig supplemented with ascorbic acid. They could not be detected along the centrilobular cell boundaries in the liver of a male guinea-pig killed on day 28 of the scorbutogenic diet although scanty deeper staining was occasionally visible in the peripheral zone.

It has been demonstrated by chemical methods that the rate of conversion of L-gulonolactone into L-ascorbic acid is strongly inhibited by *p*-chloromercuribenzoate (PCMB), and that this effect is caused by the inhibition of thiol groups in the enzyme gulonolactone oxidase³. The inhibitory effect of PCMB on gulonolactone oxidase has been confirmed by histo-

chemical studies²¹; pretreatment with PCMB of animals known to have gulonolactone oxidase in their livers eliminated detection of the hepatic enzyme by means of its staining properties. In our investigations two of the surviving guinea-pigs were withdrawn from the experiment on day 54 and treated with PCMB, which resulted in significant loss of weight and death on day 62, 8 days after the beginning of treatment. Three other survivors in the same group of guinea-pigs uniformly gained weight during this period. Two control guinea-pigs receiving the scorbutogenic diet supplemented with 50 mg ascorbic acid daily in their drinking water, and also treated with PCMB, gained weight during the 8-day period in the normal fashion (Table 2). The effect of gulonolactone supplementation of male and female guinea-pigs on the scorbutogenic diet has been described by Odumosu and Wilson²⁴. The daily administration of 30 mg kg⁻¹ of gulonolactone more than doubled life expectancy in male guinea-pigs on the diet and prolonged it in females which lived longer on average than the males. This suggests that early and continuing activation of gulonolactone oxidase (which is essential for the synthesis of ascorbic acid in several other species^{21,25}) in the presence of adequate gulonolactone is necessary, in female guinea-pigs, for prolonged survival in the absence of exogenous vitamin C.

Ascorbic acid is released from the labile stores in the buffy coat of the blood into the plasma²⁶ accounting for the initial increase in the amount of plasma ascorbic acid. It is removed from the plasma by the tissues, such as the adrenal gland, for whose continued metabolic activity it is essential. The liver requires ascorbic acid for its metabolic activity²⁷, and the concentration in the liver consequently falls as soon as the exogenous supply is stopped. After day 24 of the diet, liver synthesis of ascorbic acid rises to a level sufficient to maintain the hepatic metabolic requirements, but plasma levels continue to fall. By day 35, some escapes into the plasma from whence it is absorbed by other tissues. Female guinea-pigs in which the body weight does not fall below the initial value within the first 24 days of the diet seem to be potentially capable of synthesizing ascorbic acid. If they maintain a weight within 10% of the initial value up to the 60th day of the diet they will survive for a variable period. After day 92, body weight steadily increases in the same way as in control animals not subjected to the scorbutogenic diet. Animals showing a rapid loss of body weight after day 24 of the diet, and a subsequent decrease to below the initial weight, are potential Diers unable to synthesize sufficient ascorbic acid to satisfy essential body requirements. These animals develop scorbutic symptoms and the liver becomes pale and greasy. Deficiency of ascorbic acid impairs fat metabolism²⁸. The alterations in weight, and associated morphological changes in the liver, are among the earliest signs of uncompensated ascorbic acid deficiency in the guinea-pig²².

Release of repression of the gene, controlling the ability to synthesize ascorbic acid, may occur in some female guinea-pigs by virtue of the fact that the enzyme can be activated when there is a deficiency of exogenous vitamin C. This activation seems to occur predominantly in female guinea-pigs. The rate and maintenance of enzyme activation also determine whether enough ascorbic acid can be manufactured to enable tissues to maintain their vital metabolic functions and thus prevent otherwise inevitable death from scurvy. Variations in requirements for ascorbic acid, demonstrated by the presence of circadian and monthly rhythms²⁹⁻³¹ and by variations in ascorbic acid requirements in relation to cortisol secretion¹⁸, are associated with considerable alterations in metabolic demand for ascorbic acid on a temporal and on a tissue basis. The combined operation of the genetic, circadian and stress factors in guinea-pigs is adequate to explain the individual variation in the requirements for ascorbic acid and development of scorbutic signs, which was reported by Williams and Deason¹³.

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- ¹ Burns, J. J., Peyser, P., and Maltz, A., *Science*, **124**, 1148 (1956).
- ² Hassan, U., and Lehninger, A. L., *J. Biol. Chem.*, **223**, 123 (1956).
- ³ Chatterjee, I. B., Chatterjee, G. C., Ghosh, N. C., Ghosh, J. J., and Guha, B. C., *Biochemistry*, **74**, 193 (1960).
- ⁴ Horowitz, H. H., and King, C. G., *J. Biol. Chem.*, **200**, 125 (1953).
- ⁵ Burns, J. J., and Evans, C., *J. Biol. Chem.*, **200**, 125 (1953).
- ⁶ Isherwood, F. A., Mapson, L. W., and Chen, Y. T., *Biochem. J.*, **76**, 157 (1960).
- ⁷ Gluecksohn-Waelsch, S., *Science*, **142**, 1269 (1963).
- ⁸ Stone, I., *Perspect. Biol. Med.*, **10**, 133 (1966).
- ⁹ Todhunter, E. N., and McMillan, T. J., *J. Nutr.*, **31**, 573 (1946).
- ¹⁰ Ehme, D. A., Davey, B. L., and Todhunter, E. N., *J. Nutr.*, **58**, 281 (1956).
- ¹¹ Stubbs, D. W., and McKernan, J. B., *Proc. Soc. Exp. Biol.*, **125**, 1326 (1967).
- ¹² Sasmal, N., Kar, N. C., Mukherjee, D., and Chatterjee, G. C., *Indian J. Biochem.*, **6**, 96 (1969).
- ¹³ Williams, R. J., and Deason, G., *Proc. US Nat. Acad. Sci.*, **57**, 1638 (1967).
- ¹⁴ Grollman, A. P., and Lehninger, A. L., *Arch. Biochem. Biophys.*, **69**, 458 (1957).
- ¹⁵ Burns, J. J., *Amer. J. Med.*, **740** (1959).
- ¹⁶ Stone, I., *Amer. J. Phys. Anthropol.*, **23**, 83 (1965).
- ¹⁷ Hughes, R. E., and Hurley, R. J., *Brit. J. Nutr.*, **23**, 211 (1969).
- ¹⁸ Odumosu, A., and Wilson, C. W. M., *Brit. J. Pharmacol.*, **40**, 171P (1970).
- ¹⁹ Odumosu, A., and Wilson, C. W. M., *Brit. J. Pharmacol.*, **40**, 548P (1970).
- ²⁰ Loh, H. S., and Wilson, C. W. M., *Intern. J. Vit. Nutr. Res.*, **41**, 90 (1971).
- ²¹ Nakajima, Y., Shantha, T. R., and Bourne, G. H., *Histochemie*, **18**, 293 (1969).
- ²² Odumosu, A., and Wilson, C. W. M., *Brit. J. Pharmacol.*, **42**, 637P (1971).
- ²³ Hodges, J. R., and Hotston, R. T., *Brit. J. Pharmacol.*, **40**, 740 (1970).
- ²⁴ Odumosu, A., and Wilson, C. W. M., *Brit. J. Nutr. Proceedings* (in the press).
- ²⁵ Cohen, R. B., *Proc. Soc. Exp. Biol.*, **106**, 309 (1961).
- ²⁶ Loh, H. S., and Wilson, C. W. M., *Intern. J. Vit. Nutr. Res.* (1971).
- ²⁷ Wilson, C. W. M., *Vitamin Documentation*, **3** (Hoffman-La Roche, Basle, in the press).
- ²⁸ Ginter, E., Bobeck, P., Gerbelova, M., Ondreika, R., and Bilisics, L., *Physiologia Biochemoslovenica*, **14**, 282 (1965).
- ²⁹ Wilson, C. W. M., and Loh, H. S., *Irish J. Med. Sci.*, **2**, 396 (1969).
- ³⁰ Loh, H. S., and Wilson, C. W. M., *Brit. J. Pharmacol.*, **40**, 566P (1970).
- ³¹ Wilson, C. W. M., *Chronobiology: Proc. Intern. Soc. Study of Biological Rhythms* (edit. by Scheving, L. E., Halberg, F., and Pauly, J. E.) (Igaku Shoin, Tokyo, in the press).

Dietary Cholesterol, Sex and Scurvy in Guinea-pigs

THERE is evidence that in man the vitamin C (L-xyloascorbic acid, AA) concentration in both the plasma and leucocytes is higher in females than in males^{1,2}. On the other hand, studies with guinea-pigs receiving a controlled daily intake of AA revealed no significant sex difference between the AA concentrations in the adrenals, spleen and brain, and in the eye lens the AA concentration was significantly lower in females than in males³.

Odumosu and Wilson have recently claimed that "in times of stress, some female guinea-pigs are able to readjust their ascorbic acid metabolism and so compensate for the influence of the defective gene responsible for hypoascorbemia"⁴. Their results indicated that some female guinea-pigs could survive for up to 60 days on a scorbutogenic diet. This is in con-

trast with the generally accepted survival period of 20–30 days for male guinea-pigs given a scorbutogenic diet and would be unexpected in the light of the absence of any significant sex difference in the rates of loss of AA from the organs of guinea-pigs deprived of dietary AA⁵.

The experiment described in this note was designed further to examine the influence of sex on the survival of guinea-pigs given a scorbutogenic diet. The influence of a diet with a high cholesterol content was also examined. This was done because of recent indications of a possible metabolic relationship between AA and cholesterol^{6,7}; if AA plays an essential part in the metabolism of cholesterol then loading the body with cholesterol would be expected to shorten the survival time of guinea-pigs given a scorbutogenic diet.

Albino guinea-pigs (Dunkin-Hartley 'Pirbright' strain) of initial body weight 300–350 g were used and housed and fed as previously described⁸. The diet had the following composition: ground oats 37 g, wheat bran 35 g, dried skim milk (1% fat) 20 g, dried yeast 6.5 g, salt mixture (Glaxo Research Ltd) 1.0 g, magnesium oxide 0.5 g. Previous work had shown that male guinea-pigs given this diet without AA developed scurvy and died within 24–27 days; when supplemented with AA, normal growth was maintained⁹.

Table 1 Tissue Concentrations of Ascorbic Acid (AA) at Start of Depletion Period (after 12 Days Supplementation with AA)

	Ascorbic acid (mg 100 g ⁻¹)		
	Adrenals	Spleen	Brain
Males	214.8 ± 11.1	55.3 ± 1.7	24.6 ± 0.5
Females	226.9 ± 8.3	55.3 ± 2.1	25.6 ± 0.4

Each value is the mean with s.e. for four animals.

Thirty males and thirty females were used. All animals received 1% AA in the drinking water for 12 days immediately prior to the depletion period to produce similar saturation levels of tissue AA. On day 13 of the experimental period (= day 1 of the depletion period) four animals from each group were killed and their tissue AA levels determined (Table 1). The remaining twenty-six animals in each sex group were randomly assigned to two sub-groups of thirteen, one of which received the standard diet and the other the standard diet plus 0.3% cholesterol. The survival periods were measured (Table 2).

Table 2 Deaths of Male and Female Guinea-pigs given Scorbutogenic Diets with High or Low Cholesterol Content

Survival period (days)	High cholesterol diet		Low cholesterol diet	
	Male	Female	Male	Female
9	0	0	1	0
15	0	0	0	2
17	1	0	0	0
24	0	0	0	1
25	0	2	2	2
26	2	1	0	1
27	4	1	3	2
28	1	3	3	2
29	4	3	1	0
30	1	1	2	0
31	—	1	0	3
32	—	1	1	—
Mean survival time (d) with s.e. }	27.0 ± 0.8 (27.8)	28.2 ± 0.5 (28.2)	26.5 ± 1.5 (28.0)	25.6 ± 1.4 (27.5)

The values in parentheses are for the deaths occurring after day 20; guinea-pigs rarely die from scurvy in less than 20 days and the deaths on days 9, 15 and 17 could well have occurred from other causes.

The male and female animals had similar tissue AA concentrations at the commencement of the depletion period (Table 1) and there was no significant sex difference between the

mean survival times (Table 2). Furthermore, the additional stress introduced by the presence of cholesterol had no apparent influence on survival time. This experiment therefore does not support the claim that female guinea-pigs are less susceptible to scurvy than are males; nor does it provide evidence that there is an essential involvement of AA in the metabolism of exogenously-introduced cholesterol (although large doses of AA may nevertheless influence the metabolism of cholesterol). It may be noted that a recent population survey failed to produce any evidence of a correlation between blood AA and plasma cholesterol¹⁰.

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- ¹ Kirk, J. E., and Chieffi, M., *J. Gerontol.*, **8**, 301 (1953).
- ² Brook, M., and Grimshaw, J. J., *Amer. J. Clin. Nutr.*, **21**, 1254 (1968).
- ³ Hughes, R. E., and Jones, P. R., *Brit. J. Nutr.*, **25**, 77 (1971).
- ⁴ Odumosu, A., and Wilson, C. W. M., *Brit. J. Pharmacol.*, **42**, 637P (1971).
- ⁵ Hughes, R. E., Hurley, R. J., and Jones, P. R., *Brit. J. Nutr.*, **26**, 433 (1971).
- ⁶ Spittle, C., *Lancet*, ii, 1280 (1971).
- ⁷ Ginter, E., *Lancet*, i, 1233 (1972).
- ⁸ Hughes, R. E., and Hurley, R. J., *Brit. J. Nutr.*, **23**, 211 (1969).
- ⁹ Hughes, R. E., Hurley, R. J., and Jones, P. R., *Nutr. Repts Internat.*, **4**, 177 (1971).
- ¹⁰ Elwood, P. C., Hughes, R. E., and Hurley, R. J., *Lancet*, ii, 1197 (1970).

Mortality Rate in Male and Female Guinea-pigs on a Scorbutogenic Diet

Odumosu and Wilson¹ have reported the unexpected observation that a significant proportion of female guinea-pigs were able to survive for an unusually long period (in some cases perhaps indefinitely) on a diet free of ascorbic acid, implying that these animals could synthesize the vitamin endogenously. The literature on experimental scurvy in guinea-pigs contains, as far as we are aware, no past studies on the mortality of guinea-pigs deprived of an exogenous source of ascorbic acid in relation to sex. In experiments the animals were killed before they reached the point where death from scurvy might be expected, and the sex of animals was either not stated or male only. Nevertheless it has been assumed that both male and female guinea-pigs require an exogenous supply of ascorbic acid and that administration of an ascorbic acid-free diet would lead to the death of all animals, irrespective of sex, within 3–4 weeks. In view of the importance of the observation of Odumosu and Wilson¹ in respect of the nature of the lesion in ascorbic acid metabolism in guinea-pigs and as it might reflect on the requirements of humans for an exogenous supply of the vitamin, we felt it necessary to verify their finding.

An ascorbic acid-free diet similar to that described by Reid and Briggs², and full details of which are given below, has been in use in this laboratory for some years. This diet invariably produces, at least in male guinea-pigs, a rapid depletion of tissue ascorbic acid and a loss of appetite which

leads to a reduced food intake and fall in body weight beginning around 10 days of ascorbic acid deprivation. Characteristically, the animals exhibit a marked lassitude, poor coat condition and stiffness in the joints; they seem reluctant to place any weight on their limbs and come to adopt a typical scorbutic posture. Death generally follows 3–4 weeks after introduction of the ascorbic acid-free diet³.

For this experiment we used young albino guinea-pigs (Frant strain obtained from Mr T. Cowen, Bartlow Farm, Bartlow, Cambs., an experimental station attached to this laboratory), of both sexes. The basal, ascorbic acid-free diet used had the following composition, in parts by weight: sucrose, 103; glucose, 78; salt mixture (described below), 60; maize starch, 200; potassium acetate, 25; maize oil, 73; casein, 300; cellophane, 150; choline chloride, 2, and vitamin mixture (see below), 7.3. The salt mixture had the following composition in g kg⁻¹: calcium carbonate, 205; calcium hydrogen phosphate, 325; disodium hydrogen phosphate, 185; potassium chloride, 205; magnesium sulphate, 70; manganese sulphate, 4.5; ferric citrate, 4.35; copper sulphate, 0.375; zinc carbonate, 0.75; and potassium iodate, 0.025. The vitamin mixture consisted of the following in the proportions indicated: thiamine hydrochloride, 16 mg; riboflavin, 16 mg; pyridoxine hydrochloride, 16 mg; calcium d-pantothenate, 40 mg; cyanocobalamin, 0.05 mg; nicotinamide, 200 mg; folic acid, 10 mg; biotin, 1.2 mg; magnesium oxide, 5 g; inositol, 2 g; and 'Rovimix E' (equivalent to 250 mg α -tocopheryl acetate/g), 200 mg. Control animals received the basal diet supplemented with ascorbic acid at 2 g kg⁻¹ of diet. All animals had free access to food and water. Each animal also received by mouth, once a week, 344 μ g retinyl acetate, 5 μ g ergocalciferol, 2 mg α -tocopheryl acetate and 50 μ g 2-methyl-1:4-naphthoquinone (all dissolved in arachis oil).

All animals initially received the ascorbic acid-supplemented diet for a 2-week period of acclimatization. Animals of satisfactory growth rate were then divided as follows: a total of thirty-four male and thirty-five female animals were placed on the ascorbic acid-free diet; six male and seven female control animals continued on the ascorbic acid-supplemented diet.

Table 1 Survival Time in Male and Female Guinea-pigs Fed an Ascorbic Acid-free Diet

Survival time (days)	No. of guinea-pigs	
	Male	Female
16	0	1
17	0	0
18	0	1
19	0	4
20	1	0
21	8	5
22	6	4
23	7	3
24	3	8
25	2	6
26	2	1
27	2	1
28	2	0
29	1	1
Total	34	35

The mean survival time for both male and female groups was 23 days.

All control animals continued to gain weight throughout the experimental period. Animals fed the ascorbic acid-free diet, however, began to lose weight on average after 10 days of vitamin deprivation in the male group and

after nine days in the female group. Concomitantly, these animals began to assume the typical scorbutic appearance as described earlier. Loss of weight continued until the death of the animal. Mean weight at the start of the experiment was 244 g for the male and 242 g for the female group on the ascorbic acid-free diet; mean weight at death was 170 g for the males and 165 g for the females. As shown in Table 1, the mean survival time was 23 days in each case. At this time control animals (all of which survived) weighed around 300 g (from a mean starting weight for male and female together of 224 g). None of the animals fed the ascorbic acid-free diet survived beyond 29 days on this diet. These data concur with similar data obtained independently by Dr R. E. Hughes and his colleagues⁴.

The reason for the prolonged survival of some female guinea-pigs in the study of Odumosu and Wilson¹ in contrast to the 100% mortality within 3–4 weeks in our study and that of Jones, Hurley and Hughes⁴ is not clear. Odumosu and Wilson¹ indicate that seventeen from a total of forty female guinea-pigs could be differentiated as "potential survivors" on an ascorbic acid-free diet. Details of this diet are not provided. Conceivably it may have contained traces of ascorbic acid. If this were so, it might be possible to argue that female guinea-pigs are more adept than males in absorbing and retaining the vitamin when it is available in trace amounts only. In any case our results do not support the contention that some females can synthesize the vitamin in response to a complete absence of an exogenous source.

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¹ Odumosu, A., and Wilson, C. W. M., *Brit. J. Pharmacol.*, **42**, 637P (1971).

² Reid, M. E., and Briggs, G. M., *J. Nutr.*, **51**, 341 (1953).

³ Reid, M. E., in *The Vitamins* (edit. by Sebrell, W. H., and Harris, R. S.), **1**, 269 (Academic Press, New York, 1954).

⁴ Jones, P. R., Hurley, R. J., and Hughes, R. E., *Nature*, **242**, 521 (1973).

Rapid Generation of Whole-Body Radioautograms with Microchannel Plate Electron Image Amplifiers

WHOLE-BODY radioautography is a useful method for studying the distribution of radio-labelled compounds among all the organs and tissues of an experimental animal^{1–4}. Rapid excretion of radio-labelled drug, low energy radiation as in tritium, or low specific activity of isotopes often require weeks or months of exposure of emulsion to whole-body sections for the generation of radioautographic data. Here we describe a new simple technique for efficient, rapid generation of radioautographic images, using a microchannel plate electron image amplifier to detect electrons emitted from the surface of labelled tissue.

The microchannel plate^{5,6} (Bendix Research Laboratories) is a small, simple low-noise amplifier of electron images (Fig. 1). Each of a parallel array of glass tubes is coated on the inside with a material which conducts electricity and also emits multiple electrons upon a single electron impact. About

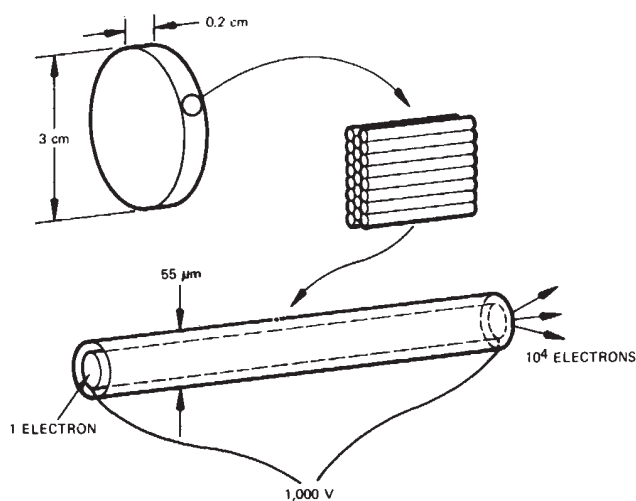


Fig. 1 Microchannel plate.

1,000 V are applied across the channel plate, and the electron gain is about 10^4 .

Specimens were placed against the input surface of the microchannel plate in a high vacuum chamber (10^{-6} Torr); vacuum was produced with an oil diffusion pump (Fig. 2). As tritium decays, electrons emerging from one tissue surface are multiplied in the active volume of a microchannel plate (MCP) and give rise to a several-thousand-fold electron emission, which is imaged onto a phosphor screen, light from which is photographed by a camera. The image quality is limited by resolution of the MCP, the noise level of the MCP amplifier, and the number of beta-particles per unit of image resolution; in our experiments a useful image was acquired in 15 min (Fig. 3B).

Thirty minutes after i.v. dosage with tritiated deoxycycline (Pfizer, 4.4 mg kg⁻¹, 5.5 mCi kg⁻¹), 50 g rats were frozen in hexane-CO₂ and whole-body sections were prepared. A whole-body section, one inch in diameter, was placed against the microchannel plate, and about 1,000 disintegrations/min were estimated in this area. The phosphor screen was photographed with 'Polaroid 57' film. Exposure times ranging from 15 to 30 min were sufficient to record enough tritium disintegrations to generate a useful image.

Fig. 3A shows the area of a whole-body section which was assayed with the microchannel plate. The corresponding MCP radioautographic image is seen in Fig. 3B. A conventional radioautogram which was generated by exposing 'Kodak No-Screen' medical X-ray film to an adjacent whole-body section for 10 weeks is seen in Fig. 3C. A disadvantage of our technique is that the microchannel plate-derived image (MCPI) is limited by the statistics of tritium decay⁷⁻¹⁰ and exhibits inferior resolution ($n \sim 30 \mu\text{m}$) to that achieved by conventional radioautography ($n = 0.2 \mu\text{m}$).

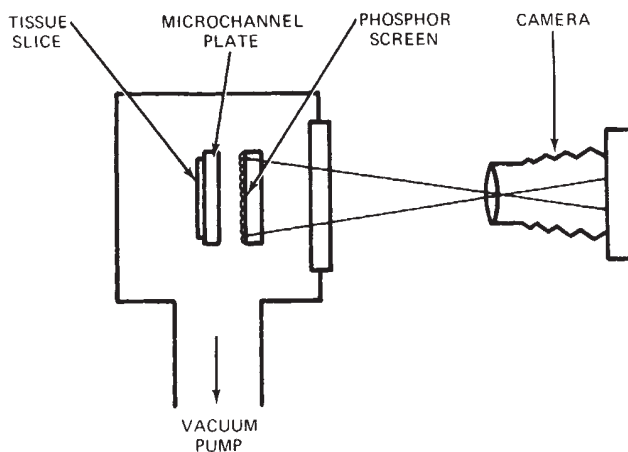


Fig. 2 Radioautography system.

In conclusion, the microchannel plate electron image amplifier has demonstrated a great advantage in speed compared with the conventional X-ray film radioautogram. The technique is simple and can be applied to whole-body sections, paper chromatograms, or any fixed solid material that can withstand high vacuum and that does not cause contamination of a vacuum system. With such samples, the microchannel plate and phosphor screen can be exposed to the atmosphere and used over and over again. With presently available components, images from a specimen up to 7.5 cm in diameter could be recorded with resolution of about $30 \mu\text{m}$. Microchannel amplifiers with unit resolutions smaller than $10 \mu\text{m}$ are now

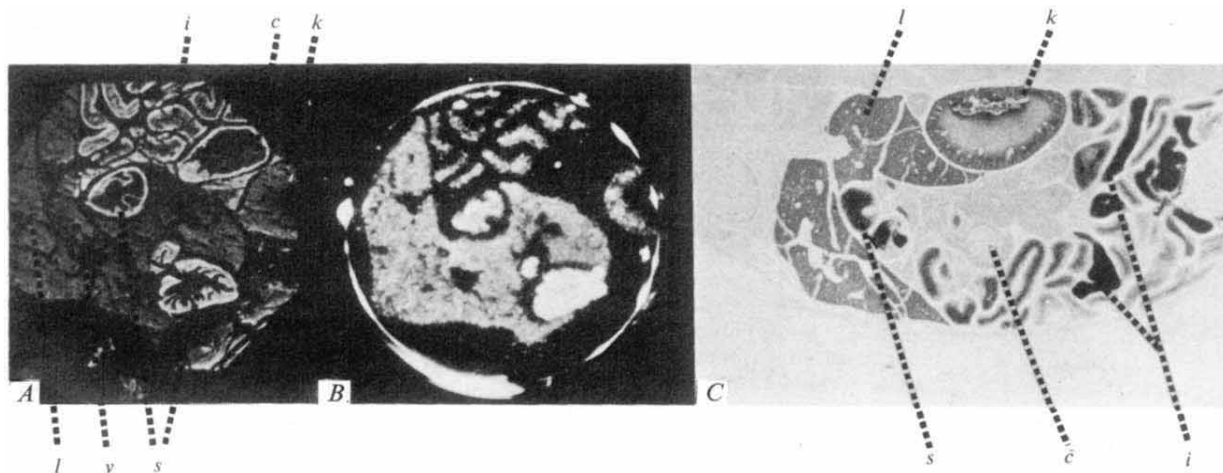


Fig. 3 A, Polaroid picture of the area of a whole-body section which was assayed with the microchannel plate. c, Colon; i, small intestines; k, kidney cortex; l, liver; s, stomach; v, hepatic vasculature. $\times 1.5$. B, Polaroid picture of microchannel plate-derived radioautographic image of tissues in A. (Light areas correspond to tissue concentrations of drug-derived ³H.) $\times 1.5$. C, Conventional radioautogram generated by exposing X-ray film to a whole-body section for 10 weeks. (Radioautogram density corresponds to tissue concentration of ³H.) $\times 1.5$.

becoming available to the commercial market and may provide yet higher resolution of radioautograms.

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- ¹ Ullberg, S., *Acta Radiol. Suppl.*, **118**, 1 (1954).
- ² Liss, R. H., Yesair, D. W., Watts, G. P., Cotton, F. A., and Kensler, C. J., *The Pharmacologist*, **10**, 154 (1968).
- ³ Liss, R. H., Cotton, F. A., and Kensler, C. J., *J. Cell. Biol.*, **43**, 81 (1969).
- ⁴ Liss, R. H., Chadwick, M., and Platz, B. B., *J. Cell. Biol.*, **55**, 157a (1972).
- ⁵ Wiley, W. C., and Hendec, C. F., *IRE Trans. Nuc. Sci.*, **NS-9**, 103 (1962).
- ⁶ US Patent 3128408 (1960).
- ⁷ Rutherford, E., and Soddy, F., *Phil. Mag.*, **4**, 370 (1902).
- ⁸ Rutherford, E., and Soddy, F., *Phil. Mag.*, **4**, 569 (1902).
- ⁹ Rutherford, E., and Soddy, F., *Phil. Mag.*, **5**, 576 (1903).
- ¹⁰ Bateman, H., *Proc. Camb. Phil. Soc.*, **15**, 423 (1910).

Inhibition of the Acute Inflammatory Response by Interferon Inhibitors

MEDIATORS responsible for the development of inflammatory reactions have been extensively studied. Much less is known about anti-inflammatory mechanisms. During the past 15 years evidence has accumulated that anti-inflammatory factors are released at the site of inflammation¹⁻¹⁰. Research in this area has been stimulated by the introduction of the carrageenin foot oedema test¹¹, which is very sensitive to endogenous anti-inflammatory factors⁷.

We observed that a synthetic polynucleotide, polyriboinosinic-polyribocytidylic acid (poly (I-C)), inhibited carrageenin and dextran-induced inflammation in the rat without influencing turpentine pleurisy (our unpublished results). Poly (I-C) has a stronger antiviral effect than is apparently accounted for by interferon production¹². As there is growing evidence that interferon and its inducers have multiple effects, we decided to investigate whether interferon inducers are involved in controlling the acute inflammatory process, and whether any activity observed could be explained by interferon production.

We studied the effect of three interferon inducers, poly (I-C), double-stranded (DS) Statolon RNA¹³ and Semliki Forest Virus (SFV), and that of a purified interferon preparation, on the acute inflammation induced in mice by carrageenin.

Six-week-old male CFLP mice weighing 20 ± 0.9 g were used. Carrageenin inflammation was induced according to the method of Levy¹⁴. For the evaluation of the inflammatory reaction, the differences between the weight of the saline and carrageenin-injected paws were expressed.

Interferon titres in mouse pooled sera were determined in tube cultures of mouse L cells using vesicular stomatitis virus (Indiana serotype). Poly (I-C) and poly (A-U), 18 Sw₂₀ and 6 Sw₂₀, respectively, were the products of Reanal (Budapest, Hungary). Purified interferon was a gift from Dr G. Bodo, Arzneimittelforschungs-Institut (Vienna, Austria), and the DS RNA of Statolon was kindly supplied by Dr W. J. Kleinschmidt, Lilly Research Laboratories (Eli Lilly and Co., Indianapolis, Indiana).

Table 1 Effect of Poly (I-C), DS Statolon RNA and Poly (A-U) on Carrageenin-induced Acute Inflammatory Response and Interferon Production in Mice

Pretreatment	Dose (mg/kg)	Increase in weight of paws caused by carrageenin (mg $\pm$ s.d.)	Inhibition of inflammatory response (%)	P	Interferon titre (units)
Untreated (15)	—	70.7 $\pm$ 10.43	—	—	0
Poly I-C (10) *	0.04	59.2 $\pm$ 4.82	16	0.05	5
	(10) 0.4	48.7 $\pm$ 5.87	31	0.0025	200
	(10) 4.0	41.2 $\pm$ 7.97	42	0.0005	1,000
	(10) 40.0	20.0 $\pm$ 4.35	71	0.0005	3,060
Statolon (6) †	0.04	64.2 $\pm$ 8.56	9	NS	5
	(6) 0.4	51.0 $\pm$ 8.32	28	0.0025	100
	(10) 4.0	46.6 $\pm$ 8.33	34	0.0005	900
Poly A-U (10) ‡	0.04	71.4 $\pm$ 9.75	0	NS	5
	(10) 0.4	68.8 $\pm$ 8.67	3	NS	20
	(10) 4.0	61.6 $\pm$ 6.58	13	NS	60

Number of CFLP mice given in parentheses. * Sw₂₀ of poly (I-C) was 18; † Sw₂₀ of poly (A-U) was 6; ‡ DS RNA of Statolon from mycophage of *P. chrysogenum*, Lot 354-1110B-62. NS, not significant. The materials were injected intraperitoneally 3 h before induction of carrageenin inflammation.

When the mice were treated with various doses of poly (I-C) and Statolon RNA 3 h before administration of carrageenin, considerable inhibition of the inflammatory response was observed (Table 1). The anti-inflammatory effect was dose-dependent and paralleled interferon production. There was no significant anti-inflammatory effect or interferon production, however, after treatment with poly (A-U). The kinetics of inhibition of acute inflammation and of interferon production were also determined (Table 2). When poly (I-C) was given simultaneously with carrageenin, the blocking effect was only moderate. If the polynucleotide was injected 1.5 or 3 h before carrageenin, however, a pronounced anti-inflammatory effect

Table 2 Inhibition of Carrageenin-induced Acute Inflammatory Response and Interferon Titres in Mice treated with Poly (I-C) and Infected with SFV

Pretreatment	Time (h) *	Increase in weight of paws caused by carrageenin (mg $\pm$ s.d.)	Inhibition of inflammatory response (%)	P	Interferon titre (units)
Untreated (15)	—	71.3 $\pm$ 11.23	—	—	0
Poly (I-C) (8) †	0	50.6 $\pm$ 6.02	29	0.0005	NT
	(8) 1.5	42.4 $\pm$ 4.58	40	0.0005	NT
	(11) 3	42.2 $\pm$ 7.97	41	0.0005	1,000
	(9) 12	43.6 $\pm$ 6.22	38	0.0005	1,000
	(9) 24	45.5 $\pm$ 14.72	34	0.005	640
	(8) 48	58.0 $\pm$ 10.6	19	0.025	5
Mouse brain suspension (10) ‡	6	69.2 $\pm$ 10.45	3	NS	NT
SFV (15) §	6	48.5 $\pm$ 7.78	32	0.0005	1,600
	(12) 12	30.7 $\pm$ 6.54	57	0.0005	7,600
	(11) 24	35.6 $\pm$ 9.3	50	0.0005	1,920
	(8) 48	45.7 $\pm$ 12.27	36	0.005	240

Numbers of CFLP mice are in parentheses. * Time of administration of interferon inducers before carrageenin injection. † Poly (I-C) (Sw₂₀ = 18) was injected intraperitoneally in a dose of 4.0 mg/kg⁻³. ‡ Normal brain tissue (2 mg) was suspended in 0.2 ml. of 0.9% saline. § 5×10^8 plaque forming units per mouse in a suspension containing 2 mg of infected brain tissue in 0.2 ml. of 0.9% saline. NT, not tested; NS, not significant. All the materials were injected intraperitoneally.

was observed. This suggests that a short period of time is required for the anti-inflammatory effect to reach its maximum, and it may, therefore, not be a direct effect of poly (I-C). The anti-inflammatory activity was long-lasting and correlated with the serum interferon titres. Ten days after adrenalectomy, the anti-inflammatory activity of poly (I-C) remained unchanged.

Infection with SFV also inhibited carrageenin-induced inflammation (Table 2). As the SFV was not highly purified, similarly prepared brain tissue of young uninfected mice was also tested and found to produce no inhibition of the inflammatory response. Six hours after infection with SFV the anti-inflammatory effect was not pronounced. Twelve and 24 h later the inhibition was strong, and the concentration of interferon in the serum was higher than in mice given poly (I-C) or double stranded Statolon RNA.

Mice were also treated with purified mouse (L cell) interferon. 100,000 plaque depressing units injected intravenously decreased oedema by only 24% (Table 3).

Table 3 Effect of Purified Interferon on Carrageenin-induced Inflammation in Mice

Group	Increase in weight of paws caused by carrageenin (mg $\pm$ s.d.)	Inhibition of inflammatory response (%)	P
Control (10)	73.0 $\pm$ 9.92	—	—
Interferon (10)*	54.0 $\pm$ 8.37	24	0.005

* The animals received 100,000 plaque depressing units of mouse L cell interferon (330,000 units mg⁻¹ protein) intravenously per animal 1 h before the subplantar injection of carrageenin. Numbers of CFLP mice are in parentheses.

Our experiments indicate that in CFLP mice, poly (I-C) and double stranded Statolon RNA exert a marked anti-inflammatory effect when studied in the carrageenin foot oedema test. This effect appears to be a reliable indicator of the interferon inducing capacity of these agents. The experiments also show that in the initial hours of SFV infection there is minimal anti-inflammatory activity, although interferon concentrations are high. The absence of a strong anti-inflammatory effect with purified interferon suggests that factor(s) other than interferon might also play a role in this phenomenon.

Kapusta and Mendelson¹⁵ reported that adjuvant arthritis was strongly suppressed by Statolon. They did not suggest that interferon was responsible for this effect, as turpentine-induced inflammation was not influenced. Our earlier (unpublished) results in rats also suggest that poly (I-C) inhibits carrageenin and dextran inflammation without influencing turpentine pleurisy. This may be due to the insensitivity of the turpentine-induced inflammatory response to endogenous anti-inflammatory products. The fact that the anti-inflammatory effect of poly (I-C) is unaffected by adrenalectomy indicates that glucocorticoids are not involved in the phenomenon.

In conclusion, our experiments suggest that, although interferon has some inhibitory effect on acute inflammation, interferon inducers are more pronounced in their anti-inflammatory activity. It will be important to determine whether the "trigger" for the anti-inflammatory effect is identical to or separate from the "trigger" for interferon production.

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- ¹ Rinaldi, T. H., *Indian J. Med. Res.*, **44**, 673 (1956).
- ² Laden, R., Blackwell, O., and Fosdick, L. S., *Amer. J. Physiol.*, **195**, 712 (1958).
- ³ DiPasquale, G., and Girerd, R. J., *Amer. J. Physiol.*, **201**, 1155 (1961).
- ⁴ DiPasquale, G., Girerd, R. J., Beach, V. L., and Steinetz, G. B., *Amer. J. Physiol.*, **205**, 1080 (1963).
- ⁵ Robinson, B. V., and Robson, J. M., *Brit. J. Pharmac.*, **23**, 420 (1964).
- ⁶ Robinson, B. V., and Robson, J. M., *Brit. J. Pharmac.*, **26**, 372 (1966).
- ⁷ Billingham, M. E. J., Robinson, B. V., and Robson, J. M., *Brit. J. Pharmac.*, **35**, 543 (1969).
- ⁸ Billingham, M. E. J., Robinson, B. V., and Robson, J. M., *Brit. Med. J.*, **2**, 93 (1969).
- ⁹ Bonta, I. L., Bhargava, N., and De Vos, C. J., *Experientia*, **26**, 759 (1970).
- ¹⁰ Kambara, T., Aimoto, T., and Hayashi, H., *Tohoku J. Exp. Med.*, **94**, 237 (1968).
- ¹¹ Winter, C. A., Risley, E. A., and Nuss, G. N., *Proc. Soc. Exp. Biol. Med.*, **111**, 544 (1962).
- ¹² Field, A. K., Tytell, A. A., Lampson, G. P., and Hilleman, M. R., *Proc. US Nat. Acad. Sci.*, **58**, 1004 (1967).
- ¹³ Kleinschmidt, W. J., Ellis, L. F., Van Frank, R. M., and Murphy, E. B., *Nature*, **220**, 167 (1968).
- ¹⁴ Levy, L., *Life Sci.*, **8**, 601 (1969).
- ¹⁵ Kapusta, M. A., and Mendelson, J., *Proc. Soc. Exp. Biol. Med.*, **126**, 496 (1967).

Electrostatic Forces in Some Hapten-Antibody Reactions

THERE is considerable interest in explaining the high specificity characterizing many of the reactions between biomolecules, in terms of interaction forces¹⁻³.

One system which readily lends itself to this type of study is a series of cross-reactions of a series of related haptens with a given antibody. Using precipitation techniques, Pressman *et al.*^{4,5} determined the relative free energies of binding for a series of substituted trimethylammonium ions with carboxylate-containing antibody sites and have estimated the distance of approach of the hapten to the carboxylate, on the basis of a model in which the full positive charge is placed on the central nitrogen. New and Richards⁶ recognized the variability of ionic charge distributions, and tried to correlate the measured free energies of association and the charge density on the hapten nitrogens, computed by the EHT⁷ molecular orbital method. As well as dangers of generalizing on the nature of this type of interaction on the basis of a series of only eight haptens, the claim of correlation seems somewhat questionable as three of the eight haptens are ordered incorrectly according to charge magnitudes. We therefore re-examined this, emphasizing two aspects of the problem of selective binding. First, we will demonstrate and explain the failure of the central nitrogen charge to account for the specificity. Second, and more important, we will attempt to determine whether the underlying cause of the specificity can be explained in terms of simple electrostatics at all.

The electrostatic interaction between a hapten and an antibody site can be calculated exactly, subject to the assumptions of a model for the site and the availability of wave functions for the interacting species. We assume, for simplicity, that the antibody reactive site on the hapten consists of a singly charged ionic group. We have calculated the IEHT⁸ charge distributions for the series of haptens. The approximate nature of the wave functions does not detract from the generality of the present work, as we are interested in relative quantities. Our second approximation, that of "hard sphere" atoms approaching to Van der Waals's distances, neglects the calculation of short-range repulsive forces. As the groups in contact remain unchanged through the series, however, this repulsion will remain approximately constant, and is unlikely to affect the relative binding energies.

With these assumptions, the electrostatic potential energy interacting with the negative point charge at site a is

$$E = \sum_i \sum_l Z_l / R_{la} - \sum_{\mu} \sum_{\nu} P_{\mu\nu} \int \chi_{\mu}(1) \chi_{\nu}(1) / r_{1a} d\tau_1 \quad (1)$$

where $P_{\mu\nu}$ is the bond order matrix, χ is an element of the atomic orbital basis and Z_l is the nuclear charge on centre l . All two and three centre integrals were evaluated exactly⁹.

For clarity a second representation of the electrostatic energy is useful. The charge distribution is partitioned into segments each of which is assigned to an atomic centre¹⁰. An infinite series of interactions between the successive electric moments on the atomic centres of the hapten with those of the antibody site will converge to the value of equation (1). The charge of the nitrogen is just the monopole moment of that atom in this atomic multipole representation. The scheme of New and Richards ignores all other atomic monopoles (including those on the methyl carbons and hydrogens which closely approach the negative charge) as well as all higher moments.

Table 1 Comparison of Free Energies Calculated from Point Charge on N with that Calculated Rigorously

Hapten (Trimethylammonium ion)	Point charge	Equation (1)
<i>p</i> -Tolyl	9.9649	71.1171
<i>m</i> -Tolyl	9.8971	69.1563
<i>o</i> -Tolyl	9.1515	70.0352
Phenyl	9.9649	70.0508
<i>p</i> -Amino phenyl	9.5582	67.3571
<i>p</i> -Amino benzyl	9.7616	69.7268

All energies in kcal mol⁻¹.

The importance of the neglected terms is indicated by Table 1, which shows that the charge on the nitrogen accounts for only a small fraction of the binding energy and that the correlation between binding energy and charge density therefore becomes meaningless.

Table 2 Relative Binding Energies Calculated (Rigorously) and Obtained Experimentally*

Hapten	Calculation	A	Experiment B	C
<i>p</i> -Tolyl	- 1.066	-0.206	-0.105	-0.105
<i>m</i> -Tolyl	+ 0.895	+0.084	+0.291	—
<i>o</i> -Tolyl	+ 0.016	+0.041	+0.077	+0.123
Phenyl	—	—	—	—
<i>p</i> -Amino phenyl	+ 2.694	+0.041	+0.077	+0.090
<i>p</i> -Amino benzyl	+ 0.324	+0.256	+0.406	+0.430

* (Refs. 4 and 5) for three different antibodies in kcal mol⁻¹.

In Table 2, the more rigorously calculated relative binding energies are shown, in some cases, to have a better correlation with experiment than given by the point charge model, but they still fall short of an overall quantitative correlation. An explanation of this lack of correlation comes from a consideration of the magnitude of the forces involved. The intrinsic electrostatic attraction between a hapten and the antibody site at Van der Waals's distance is of the order of 70 kcal mol⁻¹, while the relative binding energy is approximately 0.2 kcal mol⁻¹. In the hypothetical case of two haptens with identical charge distributions interacting with identical antibodies, where the local dielectric constant is even as high as 5, factors altering the spatial relationships between reactive sites by as little as 0.1 Å would cause changes in the binding energies between

them in excess of 0.2 kcal mol⁻¹, greater than most differences in binding energies previously reported. This suggestion is consistent with the observations made by Burgen *et al.*¹¹ on the NMR spectra of similar hapten/antibody complexes.

We would suggest that the substituents exert three major effects on the binding. The first is the influence on the electronic structure of the group adjacent to and dominating the interaction with the site. The second is on the positioning of the hapten relative to the site. Third, surface area dependent solvent effects may significantly act on bulky substituents. As shown, the first may be computed to high accuracy. Theoretical techniques for solvent forces¹² exist and are currently being studied by us. Steric relationships can be determined, at least in principle, by physical techniques like NMR¹³.

We conclude that in view of the subtle interplay of the above effects any attempt at rationalization of experiment binding data which arbitrarily disregards any of these factors can only be misleading.

We thank Professor Leonard Weiss for valuable advice, clarifying the presentation of the concepts.

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- ¹ Pullman, B., *Molecular Associations in Biology* (Academic Press, New York, 1968).
- ² Kier, L. B., *Molecular Orbital Theory in Drug Research* (Academic Press, New York, 1971).
- ³ Rein, R., *Adv. in Quant. Chem.*, **7** (1972).
- ⁴ Pressman, D., Grossberg, A. L., Dence, L. H., and Pauling, L., *J. Amer. Chem. Soc.*, **68**, 250 (1946).
- ⁵ Pressman, D., and Grossberg, A. L., *The Structural Basis of Antibody Specificity* (Benjamin, New York, 1968).
- ⁶ New, R. R. C., and Richards, W. G., *Nature New Biology*, **237**, 214 (1972).
- ⁷ Hoffman, R., *J. Chem. Phys.*, **39**, 1, 397 (1963).
- ⁸ Rein, R., Clarke, G. A., and Harris, F. E., in *Quantum Aspects of Heterocyclic Compounds in Chemistry and Biology* (edit. by Pullman, B., and Bergmann, H. D.), 86 (Jerusalem Symposia on Quantum Chemistry and Biochemistry, Israel Academy of Sciences and Humanities, Jerusalem, 1970).
- ⁹ Harris, F. E., and Michels, H. H., *Adv. in Chem. Phys.* (edit. by Prigogine, I.), **13**, 205 (1967).
- ¹⁰ Rein, R., Rabinowitz, J., and Swisler, T. J., *J. Theor. Biol.*, **34**, 215 (1972).
- ¹¹ Burgen, A. S. V., Jardetzky, O., Metcalfe, J. C., and Wade-Jardetzky, N. W., *Proc. N.A.S. (US)*, **58**, 447 (1967).
- ¹² Sinanoglu, O., in *Molecular Associations in Biology* (edit. by Pullman, B.), 427 (Academic Press, New York, 1968).
- ¹³ Jardetzky, O., and Wade-Jardetzky, N. G., *Ann. Rev. Biochem.*, **605** (1971).

Radioimmunoassay of Luteinizing Hormone Releasing Hormone

THE isolation and characterization of hypothalamic luteinizing hormone releasing hormone (LH-RH) and its subsequent synthesis have made possible the production of an antibody specific for this decapeptide.

Unlikely to be immunogenic by itself, the LH-RH was haptenized to bovine serum albumin by the carbodiimide technique¹ and injected into New Zealand white rabbits. Antibody to LH-RH appeared within 2 months (unpublished results).

Synthetic LH-RH was iodinated by the method of Hunter and Greenwood², using 5 µg of LH-RH and 1 mCi of ¹²⁵I-iodide. The labelled hormone was separated from inorganic iodide by passage through a column of 'Sephadex G-10'. Specific activities of 47–85 µCi µg⁻¹ were achieved.

The antibody to LH-RH was able to bind the labelled hormone and at a final antibody dilution of 1 in 1,200, 58% of the labelled hormone was bound.

Storage of the labelled hormone at –20° C for 4 weeks did not reduce its ability to combine with the antibody.

The binding of the labelled hormone was not affected by various polypeptides, including thyrotrophin-releasing hormone, human thyroid-stimulating hormone (TSH), ovine prolactin, ovine luteinizing hormone, human chorionic gonadotrophin, adrenocorticotrophic hormone (ACTH) and the synthetic 1–24 amino-acid chain of ACTH. These available polypeptides were present in amounts ranging from 1 to 10⁵ ng.

LH-RH and the octapeptide (TRP-SER-TYR-GLY-LEU-ARG-PRO-GLY-NH₂), lacking only the pyroglutamyl and histidyl residues from the N-terminal sequence of LH-RH, produced virtually identical decreases in the binding of the labelled hormone.

Table 1 Antibody Binding of ¹²⁵I-LH-RH in the Presence of Unlabelled LH-RH

ng unlabelled LH-RH	0	0.09	0.19	0.47	0.94	1.9	3.8	9.4
% ¹²⁵ I-LH-RH bound	21.9	20.0	19.0	16.7	14.9	13.3	10.7	2.1

0.1 ml. of LH-RH solution, 0.1 ml. of antiserum (1 in 4,500), 0.1 ml. of rabbit serum (1 in 100), 0.1 ml. of 0.05 M phosphate buffer, pH 7.2 (0.02 M EDTA, 0.15 M NaCl).

Incubated at 4° C for 48 h. Added 0.1 ml. of ¹²⁵I-LH-RH, incubated 72 h at 4° C. Added 0.1 ml. of anti-rabbit γ-globulin (donkey), 1 in 20. Incubated over 24 h at 4° C. Centrifuged at 2,000g for 30 min. Radioactivity in precipitate counted.

The addition of increasing amounts of unlabelled LH-RH produced the decreases in the binding of iodinated hormone shown in Table 1. In these conditions assays carried out in triplicate gave coefficients of variation of 1–4% at low LH-RH concentrations. The lowest concentration of LH-RH used (0.09 ng) gave a reduction of 9% in the amount of antibody-bound radioactivity relative to that found when no unlabelled hormone was present. The detection level in this assay is therefore 0.09 ng or better.

The stability of the iodinated LH-RH in the presence of serum was studied. Incubation for 4 h at 37° C of LH-RH (20 ng ml.⁻¹) either in serum, or in 0.05 M phosphate buffer, pH 7.2, or in serum containing 1,500 KIU of Trasylol did not significantly reduce binding. Further incubation for 4 days at 4° C was also without effect.

Samples of plasma obtained from twelve young, healthy females over days 10–17 of the menstrual cycle have been assayed using the procedure outlined but no measurable amounts of LH-RH have been detected. This is possibly due to the relatively insensitive nature of the present assay system or to the time of collection of the plasma samples.

The concentrations of LH-RH in the general circulation will probably be extremely low. It would seem reasonable, however, to conclude that the basis of a specific and sensitive assay for LH-RH exists. Further work is being undertaken to try and increase substantially the sensitivity of the assay in order to detect the hormone in plasma, cerebrospinal fluid, or other biological fluid.

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¹ Goodfriend, T. L., Levine, L., and Fasman, G. D., *Science, NY*, **144**, 1344 (1964).

² Hunter, W. M., and Greenwood, F. C., *Nature*, **194**, 495 (1962).

Growth of Abnormal Cells

INVESTIGATIONS have been made using the model for the growth of abnormal cells suggested by Williams and Bjerknes¹. They showed that the mathematics of the problem remained largely the same for different cell lattices, and so, for reasons of programming simplicity, consideration is limited to growths on square lattices.

Each growth begins with a single abnormal cell situated at the (approximate) centre of a lattice of normal cells. A probability $\kappa/(\kappa+1)$ (κ can be considered as the "carcinogenic advantage") is assigned to the event in which an abnormal cell divides and the subsequent abnormal daughter cell replaces one of the neighbouring normal cells. Similarly regression can occur, and there is a probability of $1/(\kappa+1)$ that one of the abnormal cells is pushed out of the layer by a normal daughter cell.

For infinitely large values of κ —that is, regression is impossible—the model is greatly simplified, and the mathematics becomes considerably more tractable. At each step of the "no-regression" model, it can be proved that when there are n abnormal cells, the number of links l_n between normal and abnormal neighbours lies in the range $(4\sqrt{n}, 2(n+1))$. It follows that if ET_n is the expected time for growth to n abnormal cells, then

$$\frac{1}{2} \ln((n+2)/2) < ET_{n+1} < m/2 - (\ln(m + \frac{1}{2}))/8 + A' \quad (1)$$

where m is the largest integer less than or equal to $\sqrt{n}$ and where the constant $A' < 0.27$.

The following improved lower bound can be obtained using a conjecture of Morgan and Walsh² and a result of Hammersley³. For large n

$$ET_n \geq K' \sqrt{n} + K'' \quad (2)$$

where $0 < K' < 1$ and $K'' < 0$.

Because inequality (2) depends upon a conjecture, it does not disprove the results of Williams and Bjerknes¹, but it does imply that one should attempt to fit a growth function of the form $ET_n = A\sqrt{n} + f(n)$, where $f(n)/\sqrt{n} \rightarrow 0$ as $n \rightarrow \infty$.

The computer used in this study was a KDF9 with 32k words of core store. This is an old machine and so it was absolutely necessary for the programming to be done efficiently. By careful addressing, we were able to obviate searching: that is, once a random number had been generated, the cells concerned were located immediately and the necessary adjustments to the listings made. For the model with no regression ($\kappa = \infty$) the computer time required for a "growth" of 10,000 abnormal cells was a little more than 1 min. This time rises quite dramatically for κ less than about 10. Thus for models in which there is regression, "growths" are limited to 1,600 abnormal cells.

The results of the simulations show that the estimated values of ET_n lie much nearer to the upper bound than the lower bound in inequality (1), and we decided to fit a function of the form

$$ET_n = A\sqrt{n} + B\ln(\sqrt{n} + \frac{1}{2}) + C \quad (3)$$

The logarithmic term was included as it occurs in both bounds and could well be an inherent characteristic of the growth function. For large n , the first term in equation (3) becomes dominant, in agreement with inequality (2).

Williams and Bjerknes¹ fitted

$$ET_n = \beta n^\alpha \quad (4)$$

to the results of their simulation. We fitted both equations (3) and (4) to our results using least squares and weights inversely proportional to the variances; the observed values and the fitted values are given in Tables 1 and 2.

Table 1 Mean Time to 10,000 Abnormal Cells for Forty Computed Growths

No. of abnormal cells	Observed values of ET_n	Fitted values of ET_n	
		W-B model (4)	D-M model (3)
25	1.65532	1.67669	1.65422
100	3.19619	3.14713	3.20236
225	4.58628	4.54862	4.60202
400	5.93916	5.90713	5.93823
625	7.24513	7.23456	7.23902
900	8.52198	8.53772	8.51718
1,225	9.79807	9.82105	9.77961
1,600	11.03331	11.08764	11.03047
2,025	12.27138	12.33978	12.27246
2,500	13.51333	13.57921	13.50744
3,025	14.73671	14.80732	14.73673
3,600	15.95674	16.02524	15.96131
4,225	17.17168	17.23388	17.18193
4,900	18.39132	18.43404	18.39919
5,625	19.60564	19.62636	19.61352
6,400	20.82619	20.81142	20.82533
7,225	22.03870	21.98971	22.03489
8,100	23.24424	23.16167	23.24249
9,025	24.44721	24.32767	24.44831
10,000	25.65223	25.48807	25.65255

Parameter values for W-B model are $\alpha=0.4542$, $\beta=0.3886$, standard error=0.0686.

Parameter values for D-M model are $A=0.23496$, $B=0.57741$, $C=-0.5049$, standard error=0.0074.

The extrapolated values of ET_n for small n ($n=1, 2, \dots, 9$) are given in Table 3. The exact values for ET_n ($n=1, 2, \dots, 9$) were calculated by considering all possible configurations, and they are included to facilitate comparisons.

The Tables show that in all cases, equation (3) provides a better fit than equation (4). Even in growths up to 10,000

abnormal cells, however, equation (4) provides a good fit to the data. The value of the exponential coefficient α shows little difference from that obtained by Williams and Bjerknes¹ when fitting over the first 1,600 abnormal cells; α is still approximately 0.45.

This phenomenon can easily be explained. It can be seen in Table 1 that the mean time to 900 cells is approximately one-third of the time to 10,000 cells and, in general, the rate of growth increases as the number of abnormal cells increases. When considering the time to n abnormal cells for individual growths, we noticed that there was always a strong positive correlation between the time to 1,600 cells and the time to 10,000 cells; for example, if the time to 1,600 cells was less than the average time, then the time to 10,000 cells was also less than the average time. Consequently, the value of α in equation (4) does not change greatly when the results are fitted up to 10,000 cells.

Table 2 Mean Time to 1,600 Abnormal Cells for 250 Computed Growths

No. of abnormal cells	Observed values of ET_n	Fitted values of ET_n	
		W-B model (4)	D-M model (3)
25	1.66050	1.67915	1.66038
100	3.18535	3.14749	3.18588
225	4.57621	4.54555	4.57776
400	5.91260	5.89983	5.91253
625	7.21631	7.22246	7.21543
900	8.50250	8.52041	8.49796
1,225	9.76662	9.79819	9.76634
1,600	11.02044	11.05894	11.02430

Parameter values for W-B model are $\alpha=0.45323$, $\beta=0.39039$, standard error=0.03088.

Parameter values for D-M model are $A=0.23790$, $B=0.51964$, $C=-0.41495$, standard error=0.00255.

To illustrate that $\alpha \rightarrow 0.5$, we fitted equation (4) to the variable $(T_n - T_{1,600})$ using least squares and weights inversely proportional to $\text{var}(T_n - T_{1,600})$. Under these circumstances $0.48 < \alpha < 0.485$. When equation (4) is fitted to the variables $(T_n - T_{3,600})$ and $(T_n - T_{400})$, α is found to be approximately 0.49 and 0.472 respectively. It seems therefore that ET_n is indeed tending towards a function of the form $A\sqrt{n}$ as n becomes large.

Table 3 Extrapolated Values of ET_n for $n=1, 2, \dots, 9$

No. of abnormal cells	Exact values of ET_n	Extrapolated values of ET_n	
		W-B model (4)	D-M model (3)
1	0.00000	0.39039	0.03364
2	0.25000	0.53448	0.25889
3	0.41667	0.64231	0.41432
4	0.54167	0.73176	0.53698
5	0.6458	0.80964	0.64003
6	0.7358	0.87939	0.72983
7	0.8160	0.94303	0.80999
8	0.8885	1.00186	0.88278
9	0.9551	1.05680	0.94972

Finite values of κ were then considered and the function (3) was again used as a fit to the observed data. Table 4 shows the parameter values obtained for both models for certain values of κ , and the standard deviations are included to illustrate that, once more, our fit seems to be better than that of Williams and Bjerknes¹.

Table 4 Parameter Values and Standard Errors for Both Models over 100 Computed Growths up to 1,600 Abnormal Cells

Value of κ	D-M model (4)				W-B model (3)		
	A	Parameter values B	C	Standard error s	α	Parameter values β	Standard error s
∞ (250 growths)	0.23790 (0.23914)	0.51964 (0.50693)	-0.41495	0.00255	0.45323	0.39039 (0.3903)	0.03088
50	0.24052 (0.24136)	0.53494 (0.52248)	-0.43151	0.00547	0.45303	0.39621 (0.3956)	0.03325
20	0.24241 (0.24486)	0.56032 (0.54703)	-0.44599	0.00885	0.45091	0.40801 (0.4040)	0.03591
10 (98 growths)	0.25097 (0.25121)	0.59442 (0.59158)	-0.47121	0.00733	0.4497	0.42778 (0.4192)	0.03561
5 (96 growths)	0.26926 (0.2663)	0.65041 (0.69740)	-0.51644	0.00526	0.44924	0.46198 (0.4554)	0.04116
3 (82 growths)	0.29815 (0.29346)	0.87830 (0.88786)	-0.90934	0.00541	0.45967	0.48626 (0.5204)	0.11298
2 (81 growths)	0.34484 (0.34777)	1.28406 (1.26880)	-1.21705	0.02291	0.44351	0.66475 (0.6505)	0.13434

"Growths" which regress to zero after one step are not included. A , B and β were fitted to $\lambda + \mu/(\kappa - 1)$ using least squares; the fitted values are given in parentheses.

If $E[T_n|\kappa]$ is the expected time to n abnormal cells for a given κ , and if we conjecture that $E[1/l_n|\kappa] \leq E[1/l_n|\infty]$, then using the theory of recurrent events the ratio $E[T_n|\kappa]/E[T_n|\infty]$ can be shown to lie in the range $[1, 1 + 2/(\kappa - 1)]$. Thus the replacement of β in equation (4) by $(\text{constant}/(\kappa - 1))$ is invalid.

The parameters A , B and β in equations (3) and (4) are obviously functions of κ , the forms of which we have been unable to establish theoretically. A , B and β have, however, been fitted, using least squares, to $\lambda + \mu/(\kappa - 1)$ and the fitted values are given in parentheses in Table 4.

In conclusion, it seems reasonable to suppose that the growth function tends to a function of the form $A\sqrt{n}$ if large enough values of n are considered. Our results are in agreement with Williams and Bjerknes and remain so for n as large as 10,000. If, however, the "transient" part of the growth function is extracted by considering variables such as $(T_n - T_{1,600})$ for each growth, we approach the "steady state" situation and α tends to 0.5.

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¹ Williams, T., and Bjerknes, R., *Nature*, **236**, 19 (1972).

² Morgan, R. W., and Walsh, D. J. A., *J. Roy. Statist. Soc.*, **B**, **27**, 497 (1965).

³ Hammersley, J. M., *J. Roy. Statist. Soc.*, **B**, **28**, 491 (1966).

Renal Blood Flow in a Diving Trained Sea Lion

THE diving reflex described for many species of animals involves a reduction in heart rate and the redistribution of blood flow to various tissues. Available evidence^{1,2} indicates that cardiac output decreases and that arterial pressure increases only modestly or remains unchanged during a dive. Flow measurements using restrained animals have tended to show cessation of flow to the kidney^{3,4} with diving. But it is well accepted that differences in these responses will exist with restrained and frightened animals as opposed to trained or free ranging animals. We investigated peripheral blood flow in a trained sea lion (*Zalophus californianus*) by measuring the renal flow

response to face immersion in water. Several months before surgery, the female sea lion was trained to place her head into a bucket, either empty or filled with water, and to remain in that position until otherwise commanded. During training, the animal wore a harness to which the necessary electronics were later attached.

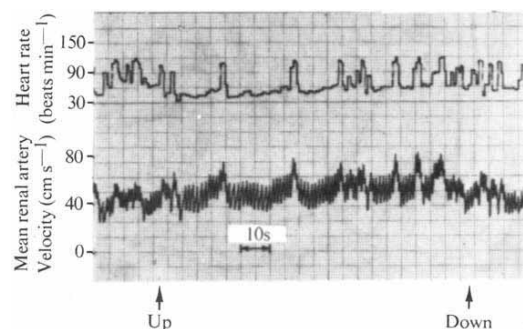


Fig. 1 Heart rate and mean renal artery flow velocity during breath holding or resting condition.

The animal was anaesthetized using a mixture of oxygen and fluothane after intramuscular injection of 0.020 mg kg⁻¹ atropine. An 8 mHz Doppler flow cuff was implanted on the left renal artery under sterile surgical conditions. Care was exercised in the dissection of the artery so that minimal damage would be done to the perivascular renal nerves. The lead wires were tunnelled under the skin to the dorsal surface of the neck at which point they were brought through the skin. The mid-line incision was closed with interrupted sutures. For the next seven days, the animal was given oral antibiotics. Training was resumed about four days after surgery, but experiments were not performed until four weeks after the operation. The Doppler flow meter used followed the design of Franklin⁵. Recordings were made of mean renal artery flow velocity and heart rate using the flow velocity pulse to trigger the cardi-tachometer. Jugular vein blood samples were obtained both pre- and post-operatively for red and white cell counts.

We show a representative portion of a control recording in Fig. 1. The resting heart rate on this occasion was 75 beats min⁻¹ and the control velocity was 45 cm s⁻¹. The "down" arrow indicates the immersion of the animal's head into an empty bucket and the "up" arrow indicates the reverse. Breath holding periods can be observed with the heart rate as

low as 36 beats min^{-1} and the mean renal artery velocity varying with the rate. Thus breath holding in this situation was part of the effect of training. The average resting heart rate was 85 beats min^{-1} and the average resting mean renal artery velocity was 48 cm s^{-1} for this animal during all of the experimental periods.

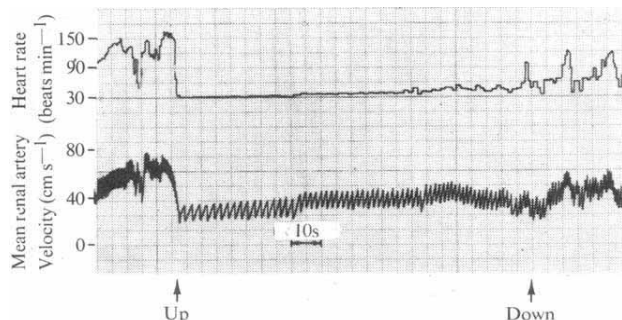


Fig. 2 Heart rate and mean renal flow velocity during face immersion for 2 min.

The record shown in Fig. 2 was obtained during an immersion in water lasting 2 min. A respiratory sinus arrhythmia occurred prior to immersion (right hand edge Fig. 2). Upon immersion a striking bradycardia began, with nadir of 30 beats min^{-1} . A reduction in mean renal artery velocity to 28 cm s^{-1} also occurred with some over-shoot in flow at the termination of the dive. The average length of 16 dives by the animal was 80 s. The mean heart rate at the end of the dive was 34 beats min^{-1} and the mean renal artery velocity was 31 cm s^{-1} , which represents an average reduction of 34% below the control value. The health status of the animal as evaluated by red and white cell counts from jugular venous blood were almost identical. Because velocity is related to volume flow by the cross-sectional area of the vessel, which is fixed in the present experiment, inferences about flow can be made.

From the studies in this unrestrained animal, it seems that inflow to the kidney does not cease but is reduced together with the cardiac output as has been shown in the duck⁶. We offer three possible explanations for the variance from previous studies: First, intrarenal redistribution of blood flow; second, species difference; third, fright. Intrarenal redistribution of flow has been demonstrated in the kidney of non-diving mammals^{7,8}. There is no way to rule out species difference; but fright is known to produce severe reaction in all animals. The record (Fig. 2) also demonstrates that there is an immediate reduction in flow, as occurs with cardiac output; thus these reductions are of neural origin. The delayed reduction in renal flow and the post-dive over-shoot of the flow response are probably related to the duration of the dive. Afferent signals of renal origin may influence the duration of diving. Therefore, oxygen may be conserved in the kidney by shunting blood around highly metabolic areas such as cortex, and simultaneously these shunts may be used to adjust vascular resistance to ensure adequate perfusion of the heart and brain and to reduce the work of the heart specifically.

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- 1 Murdaugh, jun., H. V., Robin, E. D., Miller, J. E., Drewry, W. F., and Weiss, E., *Amer. J. Physiol.*, **210**, 176 (1966).
- 2 Elsner, R. W., Franklin, D. L., and Van Citters, R. L., *Nature*, **202**, 809 (1964).
- 3 Murdaugh, jun., H. V., Schmidt-Nielsen, B., Ward, J. W., and Mitchell, W. L., *J. Cell Comp. Physiol.*, **58**, 261 (1961).
- 4 Bron, K. M., et al., *Science*, **152**, 540 (1966).
- 5 Franklin, D. L., Schlegel, W. A., and Rushner, R. F., *Science*, **134**, 564 (1961).
- 6 Johansen, K., *Acta Physiol. Scand.*, **62**, 1 (1964).
- 7 Pomeranz, B. H., Birch, A. G., and Barger, A. C., *Amer. J. Physiol.*, **215**, 1067 (1968).
- 8 Hollenberg, N. K., Epstein, M., Guttman, R. D., Conroy, M., Basch, R. I., and Merrill, J. P., *J. Appl. Physiol.*, **28**, 312 (1970).

Protection against Enteric Disease caused by *Escherichia coli* – a Model for Vaccination with a Virulence Determinant?

ALTHOUGH vaccination has assisted the control of many bacterial diseases, parenteral vaccination against enteric disease is not as satisfactory as we could wish¹. This is attributable to incomplete knowledge of both the pathogenesis of intestinal infections and the protective immune responses of the alimentary tract, with the result that vaccine development has been largely empirical. New knowledge of the specific determinants of microbial pathogenicity² provides a sounder basis for the development of effective vaccines and the following report is an example of this approach to disease control.

The pathogenesis of porcine neonatal diarrhoea involves colonization of the intestine by an enteropathogenic strain of *Escherichia coli*³ followed by production of enterotoxins⁴⁻⁷. Most strains of *E. coli* that cause neonatal diarrhoea synthesize one of two closely related protein surface antigens designated K88ab or K88ac⁸⁻¹⁰. The K88 antigen has adhesive properties that seem to be essential for the virulence of K88-positive enteropathogenic strains of *E. coli*¹¹ and enable K88-positive bacteria to proliferate in the anterior small intestine¹². Thus, the two important virulence determinants of these strains of *E. coli* are (i) K88 antigen, which enables the bacteria to attach and attain large numbers in the small intestine, and (ii) enterotoxins, which cause severe diarrhoea, resulting in dehydration and death.

From these observations, we deduced that piglets could perhaps be protected against neonatal diarrhoea either by preventing bacterial attachment to the intestinal wall or by neutralizing the effects of enterotoxins. Antibodies directed against enterotoxins may be protective^{13,14}, but protection has also been observed which seemed to be antibacterial rather than anti-enterotoxin^{15,16}. Attachment of K88-positive bacteria to piglet intestinal tissue can be demonstrated *in vitro* and is inhibited by K88 antisera¹¹. Therefore, the antibacterial activity described *in vivo* could be due to an anti-K88 factor which prevents bacterial adhesion. There is no detectable placental transfer of antibodies in the pig^{17,18}; so we postulated that K88 antibodies transmitted via the colostrum to the piglet's intestinal tract should reduce attachment of K88-positive enteropathogenic bacteria to the mucosa and render the organism less virulent.

We investigated this hypothesis by immunizing pregnant gilts with partially-purified K88 antigens, then challenging their piglets with a virulent K88-positive strain of *E. coli*. K88 antigens were extracted from two laboratory-derived K88⁺ strains [08:K27(A)⁻, K88ab:H⁻ and 08:K27(A)⁻, K88ac:H⁻] prepared by introducing the plasmids which determine

synthesis of either K88ab or K88ac antigens^{12,19} into a K-H- mutant [08:K27(A)-:H- kindly provided by Dr Ida Orskov, Statens Seruminstitut, Copenhagen] of a human peritonitis strain. No enterotoxins were detected in these two K88+ strains. K88 antigens were precipitated from cell-free homogenates by acetic acid^{9,11}; precipitates contained 98% (w/w) protein, (predominantly K88 antigen), 0.5% (w/w) carbohydrate and 1.5% (w/w) nucleic acids. Equal amounts of K88ab and K88ac antigens were incorporated in a water-in-oil emulsion²⁰, and emulsion containing 15 mg of K88 antigens was inoculated into a posterior mammary gland of four gilts 11 weeks before parturition. A saline suspension containing 30 mg of K88 antigens was given subcutaneously in the flank 10 days before parturition. Four control gilts received a primary inoculation of saline emulsion and a second injection of saline.

Approximately 1×10^{10} viable organisms of the 0149:K91 (B), K88ac(L):H10 serotype of *E. coli* were given orally to the piglets at birth before sucking¹⁵. All piglets showed clinical signs of neonatal diarrhoea within 24 h of challenge. In the four litters from non-vaccinated gilts, twenty of twenty-nine piglets died of experimental neonatal diarrhoea (END) within 72 h; the viable counts of the enteropathogenic strain in the anterior small intestine were $10^8 - 10^9$ per gramme of contents. In contrast, only four of thirty-one piglets from vaccinated gilts died of END and the clinical signs were less severe. The reduction in mortality between piglets from vaccinated and non-vaccinated gilts is statistically significant ($P < 0.01$ in a one-tailed *t* test).

It seems that K88 antibodies in colostrum were responsible for protection because (i) the vaccine was prepared from laboratory-derived strains of *E. coli* which apparently resemble the challenge strain only with respect to K88 antigens and (ii) antibodies against K88 were detected in immunodiffusion tests with colostrum from vaccinated gilts. These conclusions are supported by a report that the protective effect of antisera administered orally to piglets seemed to be directed against K88 antigens²¹. Thus, vaccination of the dam with K88 antigens provides passive protection for her piglets against challenge with a K88-positive strain of *E. coli*; protection should also be afforded against other enteropathogenic K88-positive *E. coli*. The reduced mortality among piglets from vaccinated dams suggests that the challenge strain was unable to attach and attain large numbers in the small intestine, and studies are in progress to confirm that K88 antibodies in colostrum are anti-adhesive.

Parenteral vaccination against enteric infections caused by Gram-negative bacteria is regarded as unsatisfactory because the vaccines are frequently ineffective, protection is transient or because of side-reactions associated with endotoxins. Oral prophylaxis with attenuated bacteria has potential advantages but inherent dangers are associated with the administration of live organisms. An alternative approach is to recognize the virulence determinants of enteropathogenic bacteria and to stimulate the production of protective immune responses to these determinants. Bacterial adhesive factors must be surface components and may be detectable as surface antigens. If attachment of an enteropathogenic group of bacteria to the intestinal wall is an important determinant of virulence, strains within that group should produce similar adhesive factors and hence have similar surface antigens. Strains of *Vibrio cholerae*²² and strains of *E. coli* that are enteropathogenic for infants^{23,24} or calves²⁵ attach to intestinal mucosa and common antigens have been detected in each of these groups²⁶⁻²⁸. These antigens deserve further investigation to determine if they are necessary for virulence.

Although our results refer to only passive protection, vaccination with K88 antigens could provide both a practical method for controlling porcine neonatal diarrhoea and a model for investigating a protective mechanism in the alimentary tract. This approach to microbial pathogenicity may lead to

the incorporation of concentrated, purified virulence determinants in parenterally or orally administered vaccines.

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- ¹ Wld Hlth Org. Tech. Rep. Ser., No. 500, *Oral Enteric Bacterial Vaccines*, 5 (World Health Organization, Geneva, 1972).
- ² Smith, H., in *Microbial Pathogenicity in Man and Animals* (edit. by Smith, H., and Pearce, J. H.), Twenty-second Symposium of the Society for General Microbiology (Cambridge University Press, London, 1972).
- ³ Smith, H. W., and Jones, J. E. T., *J. Path. Bact.*, **86**, 387 (1963).
- ⁴ Moon, H. W., Sorensen, D. K., and Sautter, J. H., *Amer. J. Vet. Res.*, **27**, 1317 (1966).
- ⁵ Smith, H. W., and Halls, S., *J. Path. Bact.*, **93**, 531 (1967).
- ⁶ Kohler, E. M., *Amer. J. Vet. Res.*, **29**, 2263 (1968).
- ⁷ Gyles, C. L., and Barnum, D. A., *J. Infect. Dis.*, **120**, 419 (1969).
- ⁸ Ørskov, I., Ørskov, F., Sojka, W. J., and Wittig, W., *Acta Path. Microbiol. Scand.*, **62**, 439 (1964).
- ⁹ Stirm, S., Ørskov, F., Ørskov, I., and Mansa, B., *J. Bact.*, **93**, 731 (1967).
- ¹⁰ Sojka, W. J., *Vet. Bull.*, **41**, 509 (1971).
- ¹¹ Jones, G. W., and Rutter, J. M., *Infect. Immun.*, **6**, 918 (1972).
- ¹² Smith, H. W., and Linggood, M. A., *J. Med. Microbiol.*, **4**, 467 (1971).
- ¹³ Miniats, O. P., Mitchell, L., and Barnum, D. A., *Canad. J. Comp. Med.*, **34**, 269 (1970).
- ¹⁴ Kohler, E. M., and Cross, R. F., *Amer. J. Vet. Res.*, **32**, 739 (1971).
- ¹⁵ Rutter, J. M., and Anderson, J. C., *J. Med. Microbiol.*, **5**, 197 (1972).
- ¹⁶ Smith, H. W., and Linggood, M. A., *J. Med. Microbiol.*, **4**, 487 (1971).
- ¹⁷ Kim, Y. R., Bradley, S. G., and Watson, D. W., in *Swine in Biomedical Research* (edit. by Bustad, L. K., McClellan, R. O., and Burns, M. P.), 273 (Proc. Symp. Battelle-Northwest, Seattle, USA, 1965).
- ¹⁸ Sterzl, J., Rejnek, J., and Travnicek, J., *Folia Microbiol.*, **11**, 7 (1966).
- ¹⁹ Ørskov, I., and Ørskov, F., *J. Bact.*, **91**, 69 (1966).
- ²⁰ Herbert, W. J., in *Handbook of Experimental Immunology* (edit. by Weir, D. M.), 1207 (Blackwell, Oxford and Edinburgh, 1967).
- ²¹ Smith, H. W., *J. Med. Microbiol.*, **5**, 345 (1972).
- ²² Freter, R., *Texas Reports on Biology and Medicine*, **27**, 299 (1969).
- ²³ Taylor, J., Maltby, M. P., and Payne, J. M., *J. Path. Bact.*, **76**, 491 (1958).
- ²⁴ Drucker, M. M., Yeivin, R., and Sacks, T. G., *Is. J. Med. Sci.*, **3**, 445 (1967).
- ²⁵ Smith, T., and Orcutt, M. L., *J. Exp. Med.*, **41**, 89 (1925).
- ²⁶ Neoh, S. H., and Rowley, D., *J. Infect. Dis.*, **121**, 505 (1970).
- ²⁷ Smith, H. W., and Linggood, M. A., *J. Med. Microbiol.*, **5**, 243 (1972).
- ²⁸ Bettelheim, K. A., and Taylor, J., *J. Med. Microbiol.*, **3**, 655 (1970).

Anti-bacterial Substances in Hypersensitive Responses induced by Bacteria

WHEN an avirulent fungus infects resistant plant tissue it often kills only a small number of cells and remains confined to the dead cells. This acute, very localized reaction is referred to as a hypersensitive response. When the pathogen is not an obligate biotroph, death of these cells need not explain why it does not continue to grow. In certain diseases caused by such pathogens growth is prevented because substances toxic to the pathogen accumulate at the locus of infection. Such substances are now commonly referred to as phytoalexins; in diseases of

parenchyma there is some evidence that they are largely confined to the killed cells or those immediately adjacent¹.

Plant tissues may react in a similar manner to bacterial pathogens though the process is somewhat different because individual cells are not penetrated. But with suitable techniques it can be shown that numbers of viable bacteria increase little if at all after hypersensitive response has developed; although their numbers are low, avirulent bacteria are readily recovered from tissues showing this response. Here, too, death of host cells as such does not explain the effects on multiplication of the avirulent bacteria. The phytoalexin phaseollin does accumulate in hypersensitive responses in leaves of French bean (*Phaseolus vulgaris*) caused by avirulent bacteria but this substance has little or no effect on growth of bacteria². Unknown substances accumulate in resistant bean tissues which inhibit the growth of the saprophyte *Brevibacterium linens*³. We now report on the accumulation in bean leaves of anti-bacterial substances, which probably explains why avirulent bacteria do not continue to multiply in resistant plant tissues.

French bean plants, cultivar Red Mexican, are susceptible to race 2 of the halo blight bacterium *Pseudomonas phaseolicola* but give a typical hypersensitive response to race 1, as they do to *P. mors-prunorum*, a pathogen of stone fruit trees. The saprophyte, *P. fluorescens*, causes no visible response in bean leaves.

Leaves were inoculated by injecting air spaces with suspensions of each of the bacteria; 1, 2 and 5 days later leaves were extracted with 95% EtOH. The residue after evaporation of the EtOH was taken up in water, acidified to pH 4.0 and shaken with equal volumes of ethyl acetate. Substances soluble in ethyl acetate or water were applied to 0.25 mm G nach Stahl silica gel TLC plates which were developed in methanol:chloroform (4:96 v/v), dried and then sprayed with suspensions of *P. phaseolicola* races 1 and 2, and *P. mors-prunorum* in a buffered nutrient medium (pH 7.5). The plates were incubated for 48 h in about 100% RH at about 25°C and then sprayed with a solution of tetrazolium salt (1% w/v). Areas in which bacteria failed to grow appeared white, against a pink background where bacteria had grown and in which the tetrazolium salt had been reduced by their dehydrogenases.

Three prominent areas in which bacterial growth had been inhibited had R_f values of 0.32, 0.29, 0.17 on developed plates of ethyl acetate solubles of 1, 2 and 5 day extracts of hypersensitive tissue. These areas did not appear on plates with extracts from leaves 1 or 2 days after inoculation with virulent race 2 of *P. phaseolicola* or with *P. fluorescens*. But they were found in extracts from susceptible leaves with typical water soaked lesions 5 days after inoculation with race 2.

Also five much less prominent areas of inhibition appeared in chromatograms of all extracts and two or three were sometimes found only in extracts of hypersensitive tissues. No inhibitory areas were seen on developed plates of water soluble substances.

The first three compounds were eluted from TLC plates with ethanol and purified in TLC chromatograms with benzene:acetone (50:50 v/v), methanol:chloroform (10:90 v/v), and toluene:chloroform:acetone (40:25:35 v/v). They were tested for anti-bacterial activity *in vitro* by placing impregnated filter paper disks on agar seeded with sensitive bacteria. There was no inhibition of growth when disks contained 0.5 to 5 times the amount of substances contained per gram fresh weight hypersensitive tissue 1 day after inoculation.

But when incorporated into a liquid medium (nutrients as in the agar) with a solubilizing agent (propylene glycol), and at a concentration 0.5 times that in leaves 1 day after inoculation, two of the compounds prevented growth of *P. phaseolicola* races 1 and 2, and *P. mors-prunorum* for 36 h, and the third compound did so for the whole of the 72 h incubation period. Further tests showed that the compound was bactericidal at this concentration.

The partially purified compounds had characteristic ultra-

violet spectra, gave a positive reaction with diazotized sulphanilic acid reagent, fluoresced strongly at 254 and 366 nm and showed spectral shift on addition of NaOH (2N) to ethanolic solutions. This and other evidence suggests that the compounds are phenolic; we will study their structure further.

These compounds can be obtained from hypersensitive tissue when numbers of viable bacteria stop increasing. Examination of this tissue which macroscopically appears necrotic shows that 20–33% of the cells are brown and presumably dead. These are the cells that fluoresced brightly under UV microscopy and may, therefore, be the only ones that contained the antibacterial substances. This is also suggested by the fact that these substances cannot be extracted from green tissue immediately outside brown, hypersensitive tissue. It is probable, therefore, that local concentrations of these inhibitors around bacteria that induce hypersensitive responses in cells are well in excess of those that prevent bacterial growth.

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¹ Bailey, J. A., and Deverall, B. J., *Physiol. Pl. Path.*, **1**, 435 (1971).

² Stholasuta, P., Bailey, J. A., Severin, V., and Deverall, B. J., *Physiol. Pl. Path.*, **1**, 177 (1971).

³ Lyon, G. D., thesis, Univ. London (1971).

Significance of Head Tilting in the Great Blue Heron

ONE of the problems confronting a bird that hunts for food over water is glare produced by the Sun. Several ways of reducing the interference of glare have been discussed, including both anatomical features such as dark patches around the eyes¹, coloured oil droplets in the retina² and polaroid filters³, and behavioural mechanisms such as looking through the shadow of an outstretched wing⁴. We have observed what seems to be another behavioural device for reducing the hindrance of glare.

During the course of a study of the feeding behaviour of great blue herons (*Ardea herodias*) we noticed that often when a heron that was hunting was seen head on, its head and neck were tilted quite markedly to one side (Fig. 1). This posture was often maintained for several minutes while the heron walked slowly through the water, occasionally orienting (suddenly turning the head) to or striking at prey. Thus head tilting was not simply a form of orienting to, or looking at, a potential prey item, nor was it a form of head swaying movement which might be used to obtain a parallax estimate of distance to the prey². Although the two can occur simultaneously, head tilting is distinct from head cocking, in which the bird holds its head on one side, perhaps for the purpose of monocular scanning^{5,6}.

Meyerreicks^{4,7} has apparently observed a similar phenomenon in the reddish egret (*Dichromanassa rufescens*) and little blue heron (*Florida caerulea*) and he coined the term head tilting.

We subsequently noted that head tilting was much commoner on sunny days (also noted by Meyerreicks⁴) and only occurred when the heron was facing at an angle to the Sun (that is not directly towards or away from the Sun). Further, herons almost invariably tilted their heads towards the Sun (more than eight times as often towards as away). These observations

clearly indicated that head tilting is somehow related to the Sun and presumably to the effect of the Sun on the heron's ability to catch fish (the chief prey in our study were sculpins (*Leptocottus armatus*) and flatfish (*Platyichthes stellatus*)).

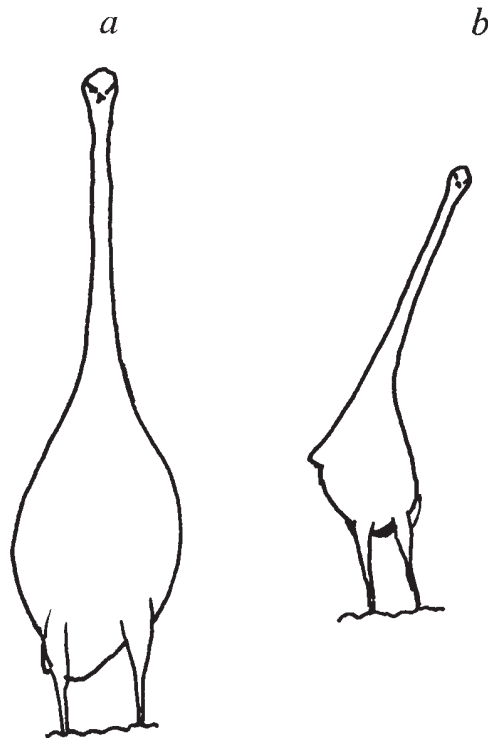


Fig. 1 Head-on views of two hunting herons traced from photographs taken (a) on a cloudy day and (b) on a sunny day. In (b) the bird is tilting its head towards the Sun.

Two types of hypothesis to explain how head tilting helps a heron hunt seemed plausible to us. (1) The prey have some reaction for avoiding predators which involves moving in a predictable direction with respect to the Sun. Thus if fish always tried to escape from herons by swimming towards the Sun (because, for example, they tried to get away from the bird's shadow), the heron might profit by leaning out towards the Sun and catching fish as they tried to escape. (2) Head tilting acts as a simple device to improve the bird's ability to see through the water and detect fish. Other hypotheses, for example, that head tilting is associated with enhancing detection of movement or perception of depth, seemed less likely to us, for they did not specifically require the bird to tilt towards the Sun. Head tilting could serve to reduce the hindrance of glare from the Sun in the following manner. If one stands in the water facing at an angle to the Sun, the Sun creates a patch of glare on the water surface just to the side of one's body that faces towards the Sun (the exact position and shape of the glare patch depend on body orientation and the height of the Sun, and the glare seems to be more distracting in turbid water). By leaning towards the Sun, one can slightly shift the glare patch further away from the area immediately in front of the body (Fig. 2). If we assume that the heron is confronted by similar problems, head tilting could serve as a device to shift the glare patch to one side and out of the normal range of reach (and therefore out of the region of water in which the bird is looking for fish). The heron would hunt in the glare-free area in front of and a little to each side of its body (Fig. 2).

If the first hypothesis is correct, the heron should strike at fish most often in the area immediately below its tilted head (to the right of the body in Fig. 2). The second hypothesis,

on the other hand, makes no particular prediction as to which direction the heron should strike in most often and one might expect the bird to strike with roughly equal frequency towards and away from the head-tilting direction as the available searching area is equal on both sides. Table 1 shows that, for a small sample of observations, herons struck at and/or caught fish with equal frequency on either side. The birds did, however, orient more often to the tilt side. Thus the data show that the herons have at best only a weak preference for hunting on the side to which they are tilting. This evidence favours the second hypothesis.

Table 1 Frequency with which Herons Struck and/or Caught Fish, or Oriented* toward Fish, towards and away from the Direction of Head Tilting

	Towards from direction of head tilt	Away from head tilt
Strike/catch	21	14†
Orient	34	18‡

* Orientation is a sudden movement of the head or neck, which presumably indicates that the bird is attending to something in the water.

† $\chi^2 = 1.4$, not significant.

‡ $\chi^2 = 4.92$, $P < 0.05$.

We examined the "glare" hypothesis further by training a hand-raised heron to hunt for dead fish in an indoor pool (4 m x 5 m, water depth 15 to 20 cm). We tested to see if it was possible to induce head tilting by means of an artificial sun (a 500 W photoflood lamp mounted on a stand at one edge of the pool 1 to 2 m above the water surface and pointing downwards). The room was completely blacked out so that the only source of glare was the "sun". These tests showed that the artificial glare did produce head tilting even in a naïve bird which had only hunted for dead fish. The bird could be induced to tilt its head to the right or left by moving the artificial sun from one side of the pool to the other; the bird always tilted towards the "sun". Head tilting was more marked if the water was made turbid. This result also supports the glare hypothesis, although it does not eliminate the

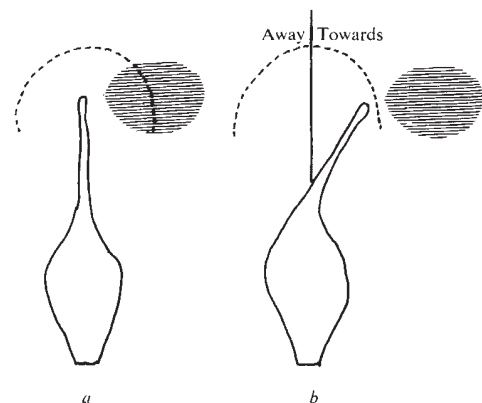


Fig. 2 A diagram of a heron as seen from above, to show how head tilting could serve to shift the position of the glare patch relative to the region of water in which the heron is looking for food. As the bird tilts its head to the right (b) (direction of Sun) the glare patch (shaded area) stays constant relative to the bird's head and shifts out of the area within the bird's reach (dotted semicircle). The bird is now holding its head more or less at the right hand limit of its reach, and can catch fish either immediately below its head or to the left. The solid line shows how we divided the bird's field of reach into "towards" and "away from" the direction of head tilting when collecting the data in Table 1.

possibility that the naïve heron had some inborn knowledge of the (hypothetical) escape response of live fish and was tilting its head for this reason.

In summary, we suggest that head tilting by great blue herons (and other related birds) serves to move the glare patch from the Sun to one side of the body, and from the area in which the bird is looking for fish. This presumably enhances the hunting efficiency of the birds in sunny weather.

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¹ Ficken, R. W., Matthiae, R. E., and Horwich, R., *Science*, **173**, 936 (1971).

² Tansley, K., *Vision in Vertebrates* (Chapman and Hall, London, 1965).

³ Pumphrey, R. J., *Biology and Comparative Physiology of Birds*, 2, Ch. xv (edit. by Marshall, A. J.) (Academic Press, New York, 1961).

⁴ Meyerreicks, A. J., *Nat. Hist.*, **71** (6), 48 (1962).

⁵ Heppner, F., *Condor*, **67**, 247 (1965).

⁶ Smith, J. N. M., thesis, Univ. Oxford (1971).

⁷ Meyerreicks, A. J., *Pub. Nuttall. Ornithol. Club*, **2**, 1 (1960).

Deep-water Mosses in Antarctic Lakes

Mosses are generally considered to be land plants although a few species are found growing submerged in streams and shallow areas of lakes. But there is now increasing evidence that certain species are able to grow successfully in deep water, often forming the dominant vegetation^{1,2}. We have made the first underwater observations on deep-water mosses growing in Antarctic lakes. A SCUBA diving survey was carried out on Signy Island, South Orkney Islands (60° 43' S, 45° 38' W) in 1971 and 1972.

Signy Island lies within the Maritime Antarctic zone³. The influence of the surrounding ocean is shown by the relatively small temperature range (monthly mean +0.8° to -10.5° C; annual mean -4.0° C), the high annual mean cloudiness (7 okta) and the low annual mean sunshine (1.5 h day⁻¹). The amount of snowfall is low (40 cm water equivalent yr⁻¹), although the frequency is high (335 day yr⁻¹). The area is extremely windy (annual mean 7.6 m s⁻¹, ref. 4).

The lakes are small and typically consist of a steep sided trough, 3–15 m deep, surrounded by a shelf formed by moraine damming. The shelf rarely exceeds 1 m in depth and is covered by scree and ground moraine, while the floor of the trough is of silt and mud broken by occasional projecting rocks⁴. The waters are soft, pH ~7, and are contaminated to a varying degree by sea spray and effluent from seal wallows and bird colonies⁵. Nitrate and phosphate levels are generally high (recorded summer range NO₃-N 190 to 19,000 µg l.⁻¹; PO₄-P 1 to 100 µg l.⁻¹). The lakes freeze to depths of 1–2 m for 8 to 12 months of each year, during which period an inverse thermal gradient develops, temperatures rising to around 1° C in the bottom layers. The waters of most lakes are very transparent but snow cover usually reduces the irradiance to a level below that thought to be necessary for photosynthesis for six months of the year, less than 1% of total visible incident

light reaching the ice–water interface. In summer the waters attain temperatures of around 5° C and are isothermal, being stirred by strong winds.

Table 1 Distribution of Deep-water Mosses on Signy Island

Moss species	Lake number					
	1	2	4	6	9	14
<i>Amblystegium</i> sp.	×	×	—	×	—	×
<i>Calliergon sarmentosum</i>	—	—	×	—	×	×
<i>Drepanocladus</i> cf. <i>aduncus</i>	—	×	×	—	—	—
<i>Drepanocladus</i> sp.	×	—	—	—	—	—
<i>Pohlia nutans</i>	—	—	—	—	—	×

The lakes have been described by Heywood^{4,5}.

No mosses were seen in Lakes 3, 5, 10, 11, 12 and 15.

* Specimen obtained by dredge in 1966 (R. I. Lewis-Smith, personal communication). Not observed by us in 1971.

There are no emergent macrophytes and, prior to our survey the vegetation was thought to consist of algae only. We found mosses growing in 6 of the 12 lakes surveyed (Table 1) and recognized two distinct communities. One, consisting of *Amblystegium* sp. or *Pohlia nutans*, was limited to the relatively few areas of rock, stones and gravel existing at depths below 2 m. The cover was discontinuous and the colonies were attached firmly to the substrate. The second community, consisting of *Drepanocladus* cf. *aduncus*, *Drepanocladus* sp. and *Calliergon sarmentosum*, occurred on mud and silt. The almost continuous cover often extended over areas of several square metres. In Lake 4, 40% of the bottom below 5 m was covered by a very luxuriant growth. *Calliergon sarmentosum* and *Drepanocladus* cf. *aduncus* occurred throughout the range of moss vegetation but the former was predominant in the shallower water and the latter at the greater depths. We observed in this lake an increase in stem length with depth, ranging from 10–20 cm at 5 m to 30–40 cm at 10 m. A similar phenomenon was observed by Bodin and Nauwerck in Lake Latnajaure, Sweden¹. In 1972 one of us (J. J. L.) found *Drepanocladus* cf. *aduncus*, *Calliergon sarmentosum* and *Campylium* sp. growing on mud below 10 m in lakes on South Georgia in the sub-Antarctic. In one lake *Drepanocladus* cf. *aduncus* was growing luxuriantly at 30 m. Lakes on this island are not subjected to such prolonged periods of thick ice cover and overlying snow as lakes on Signy.

Drepanocladus cf. *aduncus* appears to be exclusively aquatic on Signy Island but the other mosses are terrestrial species which have become adapted to the aquatic environment. *Calliergon sarmentosum*, *Drepanocladus* sp. and possibly *Amblystegium* sp. are typically hydrophytic mosses. *Pohlia nutans* is a mesophyte, becoming almost xerophytic in some habitats. This species has developed a most atypical growth form in these lakes (S. W. Greene, personal communication). The mosses seem unable to withstand ice scour and no specimens were found where this could occur. Other factors apparently controlling inter- and intra-lake distribution were silting, substrate instability and annual light regime.

Deep-water mosses are known to occur elsewhere in Antarctica. In 1957 Russian scientists dredged luxuriant growths of moss from 33–36 m depth in ice-covered lakes in the Bunge Hills area (65° 54'–66° 24' S, 100° 24'–101° 30' E) of East Antarctica⁶. They made a similar discovery in the Schirmachervatna area (70° 45' S, 11° 20'–11° 55' E) in 1962. Although these lakes remain frozen throughout each year, prevailing winds keep the clear ice free of snow. Consequently a considerable amount of incident solar radiation enters the lakes during the greater part of the year^{7,8}. The "polar night" at 70° S is less than 2 months long. Two of the mosses have

been described: *Bryum korotkeviciae*^{9,10} and *Plagiothecium simonovii*⁷.

Antarctic lakes seem to offer a more favourable physical environment for certain species than the surrounding land. In them, mosses are not subjected to desiccation, wide and rapid diurnal fluctuations in temperature, extremely low temperatures, freeze-thaw cycles and abrasion by wind driven ice crystals and granular snow—limiting factors for land vegetation¹¹. The contrast is particularly marked in the “ablation window” areas of continental Antarctica (for example, Bunker Hills and Schirmachervatna) where the land vegetation is extremely sparse and often consists only of crustose lichens and moss cushions. Further, where established, the aquatic mosses seem to receive sufficient light annually for vigorous growth.

Studies on the ecology and production of the Signy Island aquatic moss communities will be carried out by the British Antarctic Survey.

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¹ Bodin, K., and Nauwerck, A., *Schweiz. Z. Hydrol.*, **30**, 318 (1968).

² Rigler, F. H., in *Productivity Problems of Freshwaters* (edit. by Kajak, K., and Hillbricht-Ilkowska, A.) (Polish Scientific Publishers, Warsaw, 1972).

³ Holdgate, M. W., in *Biologie Antarctique* (edit. by Carrick, R., Holdgate, M. W., and Prevost, J.) (Hermann, Paris, 1964).

⁴ Heywood, R. B., *Bull. Br. Antarct. Surv.*, **14**, 25 (1967).

⁵ Heywood, R. B., *Bull. Br. Antarct. Surv.*, **18**, 11 (1968).

⁶ Korotkevich, V. S., *Inf. Byull. Sov. Antarkt. Eksped.*, **3**, 91 (1958).

⁷ Savich-Lyubitskaya, L. I., and Smirnova, Z. N., *Inf. Byull. Sov. Antarkt. Eksped.*, **49**, 33 (1964).

⁸ Heywood, R. B., *Bull. Br. Antarct. Surv.*, **29**, 35 (1972).

⁹ Savich-Lyubitskaya, L. I., and Smirnova, Z. N., *Inf. Byull. Sov. Antarkt. Eksped.*, **7**, 34 (1959).

¹⁰ Savich-Lyubitskaya, L. I., and Smirnova, Z. N., *Inf. Byull. Sov. Antarkt. Eksped.*, **17**, 25 (1960).

¹¹ Greene, S. W., and Longton, R. E., in *Antarctic Ecology* (edit. by Holdgate, M. W.) (Academic Press, London, 1970).

Increased Activity of the Peripheral Sympathetic Nervous System: Induction of Choline Acetyltransferase in the Preganglionic Cholinergic Neurone

PROLONGED increase in the activity of the peripheral sympathetic nervous system leads to augmented synthesis of tyrosine hydroxylase (E.C. 1.14.3.a) and dopamine β -hydroxylase (E.C. 1.14.2.1) in the terminal adrenergic neurone¹⁻⁶. As the induction of these key enzymes in the synthesis of the adrenergic transmitter norepinephrine is mediated by increased activity of the preganglionic cholinergic nerves², the question arises, is the augmented utilization of acetylcholine also accompanied by increased synthesis of choline acetyltransferase (E.C. 2.3.1.6), the enzyme which is responsible for the formation of acetylcholine?

We report here that treatment of rats with reserpine, which leads to a reflex increase in the activity of the preganglionic cholinergic nerves⁷, produces augmented synthesis of choline acetyltransferase in the preganglionic cholinergic neurone, as determined in the superior cervical ganglion, where the total

activity of this enzyme is confined to the preganglionic nerve terminals⁸.

Male Sprague-Dawley rats weighing 100–110 g were injected subcutaneously with 7.5 mg kg⁻¹ of reserpine and killed by a blow on the head 24 h later. Ganglia were removed and homogenized (in pairs) in 0.5 ml. of ice-cold 0.005 M Tris buffer (pH 7.4) containing 0.1% Triton X-100. The homogenates were centrifuged at 27,000g for 20 min at 0–5° C. Choline acetyltransferase activity was determined in the supernatant by the method of Fonnum⁹, with modifications described in detail elsewhere¹⁰. Proteins were assayed according to the method of Lowry *et al.*¹¹.

Reserpine treatment resulted within 24 h in a marked increase of the *in vitro* activity of choline acetyltransferase, which amounted to 152% of controls in the superior cervical and to 161% in the stellate ganglion. Increased levels still persisted after 48 h (Table 1). If enzyme preparations of controls and reserpine treated animals were combined in various proportions the activity was always additive, that is, corresponding to the sum of the two components. This finding suggests that the increased activity does not result from the disappearance of an inhibitor or the formation of an activator, but rather from an increase in enzyme protein.

Table 1 Increase of Choline Acetyltransferase Activity in Ganglia after Treatment of Rats with Reserpine

	Superior cervical ganglion		Stellate ganglion	
	nmol acetylcholine h ⁻¹ per pair	nmol acetylcholine h ⁻¹ mg ⁻¹ protein	nmol acetylcholine h ⁻¹ per pair	nmol acetylcholine h ⁻¹ mg ⁻¹ protein
Controls	3.60 ± 0.28	16.7 ± 1.0	2.78 ± 0.24	13.9 ± 1.6
Reserpine 24 h	5.12 ± 0.62 *	25.4 ± 1.4 *	4.30 ± 0.34 *	22.3 ± 1.9 *
Reserpine 48 h	4.92 ± 0.58 *	26.8 ± 3.5 *	4.71 ± 0.61 *	25.2 ± 2.2 *

Animals were injected subcutaneously with 7.5 mg/kg reserpine and killed after the time interval indicated. The concentration of choline in each assay was 10 mM, that of acetyl-CoA 0.1 mM. Each value represents the mean ± s.e. of eight duplicate determinations.

* $P < 0.001$, compared with controls.

To substantiate this assumption the effect of cycloheximide, an inhibitor of ribosomal protein synthesis^{3,12,13}, was studied. In these experiments determination of choline acetyltransferase activity was confined to the superior cervical ganglion (Table 2). The animals were treated with 0.9 mg kg⁻¹ of cycloheximide every 6 h. The first dose was given at the same time as reserpine. The choline acetyltransferase activity was significantly ($P < 0.025$) lower than that in animals treated with reserpine alone but did not differ significantly ($0.4 > P > 0.3$) from that of animals treated with cycloheximide alone. The small but statistically significant ($P < 0.05$) increase in choline acetyltransferase activity after treatment with cycloheximide alone is probably due to a stress exerted by the drug and to the fact that the relatively low dose, which could not be increased without killing the animals before 24 h, does not completely inhibit protein synthesis³. The fact that the protein level is not significantly higher after reserpine treatment ($0.4 > P > 0.3$) shows that the increase in choline acetyltransferase activity is not a manifestation of generally increased protein synthesis but represents a specific effect as previously shown for the induction of tyrosine hydroxylase¹ and dopamine β -hydroxylase⁶ in the terminal adrenergic neurone. In preliminary experiments an increase of choline acetyltransferase activity in superior cervical and stellate ganglia, very similar to the increase after reserpine treatment, was observed after exposure of rats to a relatively short (2 h) but intense stress by swimming to exhaustion.

Goldberg and Welch¹⁴ have reported a marked increase in choline acetyltransferase activity of mice adrenals on intermittent psychosocial stimulation for 10 days¹⁴. Although the mechanism underlying the increased enzyme activity was not investigated, it seems reasonable to assume that an increased synthesis of new enzyme protein also takes place in these

Table 2 Inhibition of Reserpine-induced Increase of Choline Acetyltransferase Activity in Superior Cervical Ganglia by Cycloheximide

	Choline acetyltransferase nmol acetyl- choline h ⁻¹ per pair of ganglia	nmol acetyl- choline h ⁻¹ mg ⁻¹ protein	Protein per pair of ganglia (μg)
Controls	3.46 ± 0.29	14.4 ± 1.1	241 ± 7
Reserpine	5.39 ± 0.32*	22.7 ± 0.7*	253 ± 10
Cycloheximide	4.13 ± 0.24†	18.4 ± 0.8†	224 ± 6
Reserpine + cycloheximide	4.28 ± 0.30‡	19.4 ± 1.2‡	227 ± 13

Animals were injected subcutaneously with 7.5 mg kg⁻¹ reserpine 24 h before they were killed. Cycloheximide (0.9 mg kg⁻¹) was injected every 6 h for the same length of time. The concentration of choline in each assay was 10 mM, that of acetyl-CoA 0.1 mM. Each value represents the mean ± s.e. of ten duplicate determinations except for the combined reserpine and cycloheximide treatment (*n* = 5).

* *P* < 0.001, compared with controls.

† *P* < 0.05, compared with controls.

‡ *P* < 0.025, compared with reserpine alone.

experimental conditions. Thus it appears that neuronally mediated induction of enzymes is not confined to the biosynthetic pathway of norepinephrine, but that a similar adaptation to increased transmitter utilization takes place in the preganglionic cholinergic neurone.

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- ¹ Mueller, R. A., Thoenen, H., and Axelrod, J., *J. Pharmac. Exp. Ther.*, **169**, 74 (1969).
- ² Thoenen, H., Mueller, R. A., and Axelrod, J., *J. Pharmac. Exp. Ther.*, **169**, 249 (1969).
- ³ Mueller, R. A., Thoenen, H., and Axelrod, J., *Molec. Pharmacol.*, **5**, 463 (1969).
- ⁴ Thoenen, H., *Nature*, **228**, 861 (1970).
- ⁵ Molinoff, P. B., Brimijoin, W. S., Weinshilboum, R., and Axelrod, J., *Proc. US Nat. Acad. Sci.*, **66**, 453 (1970).
- ⁶ Thoenen, H., Kettler, R., Burkard, W., and Saner, A., *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **270**, 146 (1971).
- ⁷ Iggo, A., and Vogt, M., *J. Physiol., Lond.*, **150**, 114 (1960).
- ⁸ Thoenen, H., Saner, A., Angeletti, P. U., and Levi-Montalcini, R., *Nature New Biology*, **236**, 26 (1972).
- ⁹ Fonnum, F., *Biochem. J.*, **115**, 465 (1969).
- ¹⁰ Oesch, F., Otten, U., and Thoenen, H., *J. Neurochem.* (in the press).
- ¹¹ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).
- ¹² Young, C. W., Robinson, P. F., and Sacktor, B., *Biochem. Pharmacol.*, **12**, 855 (1963).
- ¹³ Trakatellis, A. C., Montjar, M., and Axelrod, A. E., *Biochemistry*, **4**, 2065 (1965).
- ¹⁴ Goldberg, A. M., and Welch, B. L., *Science, NY*, **178**, 319 (1972).

Late Precambrian Microfossils: a New Stromatolitic Biota from Boorthanna, South Australia

OF the four dozen fossiliferous Precambrian sediments now known¹, only a very few contain diverse microfossil assemblages preserved *in situ* on which inferences of ecologic setting and evolutionary status can be based reliably. The time gaps between these deposits are enormous, commonly approaching or exceeding a duration equivalent to that of the entire

Palaeozoic. Present inferences regarding the course of early evolutionary development are therefore highly speculative. There is obvious need for the detection of additional evidence with which to evaluate such inferences and to fill in the skeletal picture that has emerged in recent years. We report here the discovery of a well preserved, stromatolitic microbiota from the late Precambrian of Boorthanna, South Australia, that should contribute measurably toward this goal.

In organization, mode of preservation, and ecologic setting, the newly discovered microbiota seems comparable to the benchmark Precambrian assemblages of the Gunflint², Beck Spring³ and Bitter Springs cherts⁴. Like these assemblages, this microflora comprised a mat-building photosynthetic community that was permineralized by silica near the sediment-water interface in a shallow, presumably marine environment. The Boorthanna community, however, is unusual both in its abundance and its fidelity of preservation. Moreover, it is exceptionally diverse, apparently including bacteria, filamentous, unicellular and colonial blue-green algae, and several types of unicellular and possibly colonial eukaryotes. As perhaps the most diverse and abundant of the few stromatolitic microfloras now known, the assemblage provides an unusual opportunity to investigate the relative importance of biotic and environmental factors in the development of stromatolite morphology, a matter crucial to the proposed use of stromatolites as biostratigraphic indicators⁵. In a broader context, the location of this late Precambrian microflora, midway between the major portion of the Adelaide Geosyncline to the south and the Amadeus Basin to the north, and the fact that microfossils and stromatolites occur in sediments of similar age in these two adjacent regions^{4,6,7}, suggest that the Boorthanna deposit could provide important evidence bearing on the difficult problem of interregional biostratigraphic correlation in the Precambrian. As for its evolutionary implications, this approximately 1,000 m.y. old microflora, roughly intermediate in geologic age and biotic composition between the Beck Spring and Bitter Springs assemblages, seems to represent a stage in biological history characterized by "modernization" of the cyanophytic flora⁴, diversification of microscopic eukaryotes, and, possibly, emergence of the evolutionarily important process of eukaryotic sexuality⁸. The Boorthanna fossils should yield fresh insight into the composition and status of the early biota during this active evolutionary stage, a stage apparently transitional between the development of primitive, unicellular eukaryotes and the very late Precambrian emergence of multicellular, megascopic plants and animals.

The microflora is preserved in black, laminated cherts occurring within an unnamed dolomitic formation in the Peake and Denison Ranges of South Australia, an uplifted, northern extension of the Adelaide Geosyncline. Samples were collected near Boorthanna about 2 km east of the William Creek-Oodnadatta road, 460 km north-northwest of Port Augusta. The fossiliferous horizon is situated 893 m beneath the top of the formation and 1,233 m beneath the base of a tillite equivalent to the late Precambrian Sturt Tillite of the southern Adelaide Geosyncline. The unnamed formation is apparently correlative with the Adelaidean Skilloalee Dolomite, a correlation suggesting an age of about 1,000 m.y. for the formation^{6,9}.

The Boorthanna cherts are remarkably fossiliferous, commonly containing more than 10,000 microfossils cm⁻³. The fossils range from brownish amber to dark brown and can be freed from the chert by hydrofluoric acid maceration. The orientation (Fig. 1*d*) and great length (Fig. 1*a*) of the organically preserved filaments and the occurrence of the assemblage within stratiform and *Collenia*-type stromatolites indicate that the Boorthanna organisms constituted a mat-building microbiological community that was permineralized by silica *in situ* near the sediment-water interface.

The more than two dozen types of microorganisms recog-

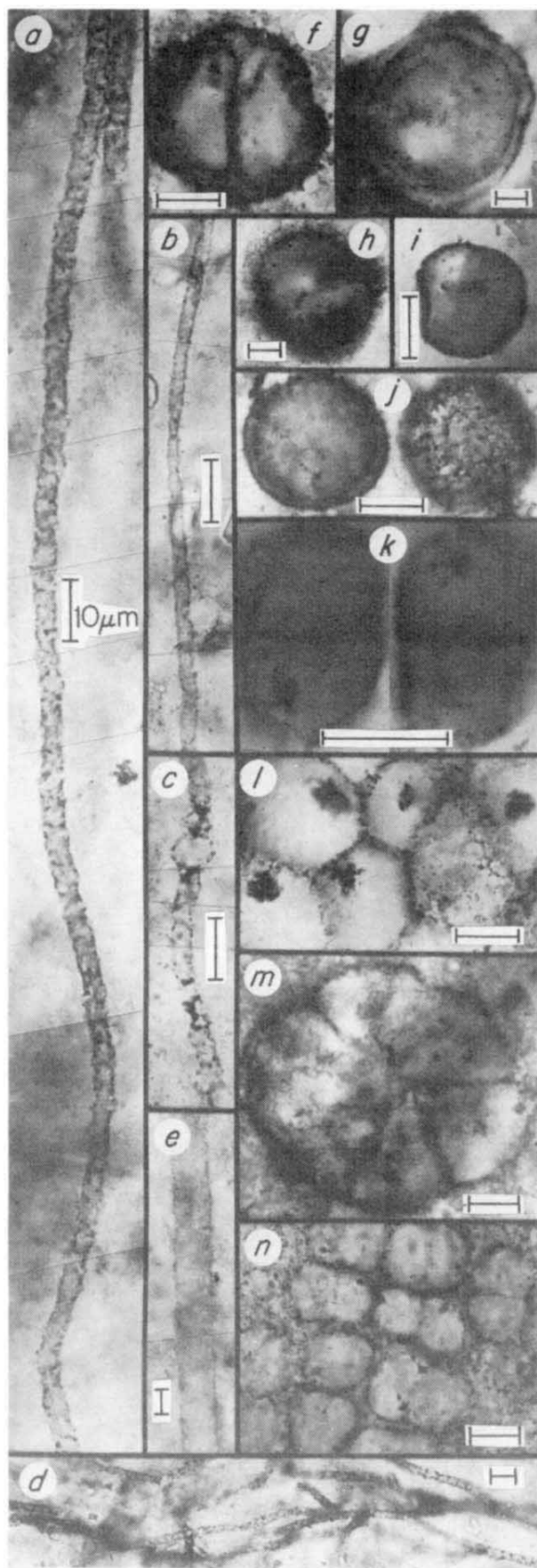


Fig. 1 Optical photomicrographs showing microfossils in thin sections of late Precambrian chert from Boorthanna, Australia. Composite photomicrographs are shown in *a-c* and *e*; specimens in *j* and *k* are shown at two focal depths; lines for scale, 10 μ m.

nized in the deposit will be described formally after additional study. Here we divide the assemblage into five broad categories: cellular filaments, nonseptate filaments, solitary unicells, colonial unicells, and unicells containing organelle-like bodies.

Cellular filaments (Figs. 1*a-d*) are uniseriate, unbranched and range from 0.5 to 6 μ m in diameter. The narrowest filaments are probably bacteria. Broader filaments seem comparable to nostocalean blue-green algae, a comparison strengthened by the occurrence of enlarged, intercalary, heterocyst-like cells (Fig. 1*c*).

Nonseptate filaments (Fig. 1*e*) are broad and thin-walled; like comparable forms from the Gunflint (*Animikiea*²) and Bitter Springs assemblages (*Siphonophycus*⁴), they seem to be empty sheaths that originally enclosed cellular blue-green algal trichomes.

Solitary unicells (Figs. 1*g-j*) range from 3 to 60 μ m in diameter and have psilate or granular surfaces. Most resemble chroococcacean cyanophytes; many, however, are of uncertain affinities (for example, Fig. 1*g, j*).

Colonial unicells, morphologically quite similar to living blue-green algae, occur both in rounded aggregations (Fig. 1*m*; compare *Myxosarcina*, Pleurocapsaceae) and in tabular groupings one cell thick (Fig. 1*n*; compare *Merismopedia*, Chroococcaceae).

A large number of unicells, commonly occurring in colony-like clumps (Fig. 1*l*), contain granular, spheroidal, organic bodies that may represent preserved organelles (Fig. 1*f, k, l*). Similar organelle-like bodies occur in permineralized Phanerozoic plants¹⁰ and in unicells of the Bitter Springs and Beck Spring assemblages. Although further investigations are needed to better define the nature of these bodies, their occurrence in the Boorthanna assemblage seems consistent with recent studies^{3,4,8} apparently indicating that unicellular eukaryotes (green and red algae) were well established during the late Precambrian and suggests further that primitive eukaryotic colonies may have been extant as early as 1,000 m.y. ago.

We thank Dr H. Wopfner, South Australia Department of Mines, for collecting the chert samples and for providing data on the geologic setting of the fossiliferous locality ("North Boorthanna Section" APD 5, measured by the French Petroleum Co., Aust., 1963). Supported by the US National Science Foundation and, in part, by the US National Aeronautics and Space Administration.

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- ¹ Schopf, J. W., *Biol. Rev. Camb. Phil. Soc.*, **45**, 319 (1970).
- ² Barghoorn, E. S., and Tyler, S. A., *Science*, **147**, 563 (1965).
- ³ Cloud, jun., P. E., Licari, G. R., Wright, L. A., and Troxel, B. W., *Proc. US Nat. Acad. Sci.*, **62**, 623 (1969).
- ⁴ Schopf, J. W., and Blacic, J. M., *J. Paleontol.*, **45**, 925 (1971).
- ⁵ Cloud, jun., P. E., and Semikhatov, M. A., *Amer. J. Sci.*, **267**, 1017 (1969).
- ⁶ Preiss, W., *Quart. Geol. Notes*, **38**, 3 (Geol. Survey South Australia, 1971).
- ⁷ Schopf, J. W., and Barghoorn, E. S., *J. Paleontol.*, **43**, 111 (1969).
- ⁸ Schopf, J. W., Haugh, B. N., Molnar, R. E., and Satterthwait, D. F., *J. Paleontol.*, **47** (in the press).
- ⁹ Thomson, B. P., *J. Geol. Soc. Australia*, **13**, 203 (1966).
- ¹⁰ Gould, R. E., *Amer. J. Bot.*, **58**, 239 (1971).

BOOK REVIEWS

Antarctic Earth Science

Antarctic Geology and Geophysics. Edited by R. J. Adie. (Symposium on Antarctic Geology and Solid Earth Geophysics, Oslo, August 1970.) Pp. 876. (Universitetsforlaget: Oslo, 1971.) £25.35.

THIS is the second massive international symposium volume on Antarctic earth science to appear within a decade. It is a work of major importance and must find a place on the shelves of every geological or geophysical library that pretends to global concern. Delving into the volume it is a joy to see the solemn words of the Antarctic Treaty of 1959 once again translated into very tangible scientific results. Although both earth and life sciences have drawn rich rewards from Antarctica since it was agreed to make the continent an international scientific laboratory, geologists have led the way in painting a coherent picture of the area without regard to former national spheres of interest. The first symposium on Antarctic geology was held in Cape Town in 1963. It was organized by the Scientific Committee on Antarctic Research of the International Council of Scientific Unions and sponsored by the International Union of Geological Sciences. The 1970 symposium held in Oslo was similarly organized and sponsored and the proceedings of both meetings were edited with painstaking thoroughness by R. J. Adie. The earlier volume weighed 2 kg and cost £11. The present volume weighs 3 kg and at £25.35 has unfortunately priced itself out of the market for individual scientists. It contains 126 papers covering almost all the significant advances made since science began to loom larger than politics in Antarctica. The Cape Town symposium was organized with group sessions based on separate disciplines within the earth sciences. As a result too many studies appeared not only geographically but also thematically isolated. This situation was remedied at the Oslo meeting which was instead based on regional studies. The evident and rather new-found awareness among geologists of what they stand to gain from solid earth geophysics on a conti-

nent 99 per cent hidden beneath ice is one of the more exciting rewards of a regional approach. While the Cape Town volume contained one short paper on the "importance of Antarctica in the hypothesis of continental drift", almost every author in the present volume shows an implicit if not an explicit awareness of the significance of his findings in a Gondwanaland context.

A number of invited review papers on specific topics form nuclei around which original contributions are arranged. While the reviews are of widely varying depth and significance, they are authoritative and most were up-to-date in 1971. They include D. H. Griffiths and P. F. Barker on marine geophysical investigations in the Scotia Sea, R. J. Adie on recent advances in the geology of the Antarctic Peninsula, P. J. Barrett and others on the Beacon Supergroup, E. H. Colbert on Triassic tetrapods, C. Craddock and also G. E. Grikurov and others on tectonics, R. D. Adams on dispersion-wave studies, A. Ya Krylov on geochronology, M. G. Ravich on regional metamorphism of the crystalline basement, D. S. Soloviev on platform magmatic formations, P. E. Calkin and R. L. Nichols on Quaternary studies, G. de Q. Robin on radio-echo sounding, D. E. Hayes and W. C. Pitman on marine geophysics, and M. Girod and J. Nougier on volcanism in the sub-Antarctic islands. The relative interest in problems of particular regions can be judged from the fact that there are twenty-four papers and two abstracts on the Scotia Arc and Antarctic Peninsula, sixteen papers and one abstract on western Antarctica, twenty-two papers and one abstract on the Transantarctic Mountains, thirty-seven papers on eastern Antarctica, five papers and four abstracts on the southern ocean, eight papers on the sub-Antarctic islands, and four papers on continental drift. As with most volumes resulting from symposia attended by the leading figures in the subject, there is some republication of material presented earlier in journals such as *Nature* and *Science*. This is perhaps justified in that the fuller versions now presented are in context and appear in association

with other contributions relevant to an understanding of regional relationships. Indeed so comprehensive is the subject coverage of this and the earlier volume that only Antarctic specialists will need anything else on their shelves. By the time a further symposium volume appears, reconnaissance geology and systematic mapping at the smaller scales will be complete and new chapters in the history of the continent will be based on the results of continued palaeomagnetic studies, much deep sea drilling and to a lesser extent sub-ice drilling. It is becoming increasingly clear that although the rock surface of almost the whole continent lies beneath an ice sheet which in parts reaches 5,000 m in thickness, we already have techniques that can be used to delineate the main structural and lithological boundaries beneath the ice. Seismic refraction, magnetic, gravity, and radio-echo sounding surveys, much employed singly, now give promise of a vastly increased yield when brought together.

In discussing a volume of 876 pages the reviewer can only draw specific attention to a few arbitrarily selected topics. Recent reconnaissance mapping appears finally to have located the boundary between the Andean structural units of the Antarctic Peninsula fold system and the Marie Byrd Land ranges, the latter being interpreted by F. A. Wade and J. R. Wilbanks as displaced fragments of the eastern Antarctic shield. However, the history has been much complicated by orogenic processes and volcanism. Some forty volcanoes have now been identified in Antarctica, and while most show few signs of recent activity, Deception Island has experienced a series of spectacular eruptions in 1967, 1969, and by coincidence also in August 1970 while the symposium was in progress in Oslo.

There has been much recent and detailed exploration of the Transantarctic Mountains, which are now believed to extend right across the continent as far as 73°S, 02°W in Dronning Maud Land. D. H. Elliott and others discuss the relationship of the Beacon Supergroup rocks to Gondwana sequences. Stratigraphical similarities include Palaeozoic tillites, dark shales overlying Palaeozoic glacial deposits,

Permian coal measures, and basalts capping the succession. The best-known palaeontological similarity is widespread *Glossopteris* flora. Until 1967 the only vertebrates recorded were Devonian and Jurassic fish remains. Tetrapod remains, which are an important part of the palaeontology of Gondwana sequences, were not found in Antarctica until December 1967. E. H. Colbert describes the exciting discovery by P. J. Barrett and others, at Coalsack Bluff near the Beardmore Glacier, of an abundant tetrapod fauna similar to the Lower Triassic *Lystrosaurus* Zone fauna of the Upper Beaufort Series, South Africa.

There is plenty of work left for the next generation. Still unsettled is the age of the start of uninterrupted continental glaciation, and whether or not the depressed continental shelf of Antarctica is due to a former greater ice loading. Until quite recently the ice sheet was regarded as a strictly Pleistocene phenomenon. Now R. H. Rutford and others conclude on the basis of glacial deposits overlain by dated volcanics that the Jones Mountains area was undergoing continental glaciation 7 million years ago, while LeMasurier suggests that an ice sheet of substantial thickness has existed continuously in western Antarctica since Eocene time. It is an intriguing subject and we can eagerly anticipate the next instalment.

The book is beautifully printed on A4 size paper, the line drawings are explicit and clear, there are several hundred good photographs and there is an index. Misprints are few by any standard. When we consider that the contributing authors came from twelve different countries (only five of them English-speaking), and that the volume was printed in English in Norway, surely the editor and all concerned deserve our congratulations and thanks for a magnificent achievement.

CHARLES SWITHINBANK

Liquid Metals

An Introduction to the Theory of Liquid Metals. By T. E. Faber. Pp. xiii + 587. (Cambridge University: London, November 1972.) £12; \$37.50.

THE study of liquid metals in a structured way began twelve years ago with the application of the pseudopotential concept, providing a theoretical framework for the subject which has remained ever since. A first international conference devoted to the properties of liquid metals was held in 1966 and a second in 1972; it is a measure of the continuing momentum of the subject that a third is planned for 1976. During this time several reviews have appeared and a concise monograph (March,

N. H., *Liquid Metals*, Pergamon, London, 1968) but there has been a need for a more extensive exposition. This gap is well filled by Dr Faber's book. The author has been active in the field throughout. As a result the book quite properly contains a good deal of his own original work, some of it not previously published, and is liberally sprinkled with critical comment and suggestions for further work. The level is such as to appeal particularly to experimental physicists whether new to the field or not. Mathematical arguments are kept at a relatively simple level and are fairly fully presented.

The text consists of six chapters, the first devoted to a discussion of nearly free electrons (NFE) on which the effect of the ions is calculated by perturbation methods using a pseudopotential. This is basic to the rest of the book in which the recurring theme is the extent to which an NFE model is applicable to real liquid metals. This governs in part selection of material; liquid transition metals and liquid semiconductors are dealt with very briefly since neither can be included under an NFE umbrella.

There follow two chapters on structure and atomic motions. A discussion of the pair distribution function and its Fourier transform, the liquid structure factor, includes a critical survey of experimental data and of progress towards solution of the central problem of relating the distribution function to a pair potential. The discussion of atomic motions centres round interpretation of the diffusion coefficient, particularly the light recently thrown on the detailed nature of diffusion by molecular dynamics, and progress in the difficult business of extracting details of atomic motions from neutron scattering data.

The next two chapters return to the conduction electrons, first turning to the theoretical problem of electron eigenfunctions in disordered systems, and the experimental help we can expect from techniques such as the Compton effect. This leads the way for a discussion of electron transport, both d.c. and at optical frequencies, where the remarkable successes of the NFE model appear in profusion and discrepancies of detail even begin to be understood. The final lengthy chapter extends the theoretical ideas to a treatment of alloys and surveys the vast pool of available experimental data.

A successful innovation is the use of a prologue preparing the reader for the argument in each chapter; this will surely be found useful and the practice should be commended to other authors. The book is furnished with a useful collection of references; fittingly some two-thirds of them (about 500) relate to the period 1965–72 which has seen the bulk of the activity in this relatively young subject. E. F. W. SEYMOUR

Protobiogenesis

Molecular Evolution and the Origin of Life. By Sidney W. Fox and Klaus Dose. Pp. xi + 359. (W. H. Freeman: San Francisco and Reading, 1972.) \$16.

THIS volume comes riding in on the crest of a great wave of new books on the origin of life, but it is very much more than just "one of the rest". Many of us have for a long time been eagerly waiting for its appearance. The authors are both distinguished practitioners of a small band of experimentalists who have been conspicuously successful in the thermal synthesis of proteinoid polypeptides. Sidney Fox has for many years championed a model in which proteinoid microspheres are regarded as "protocells", and he has shown that his microspheres, synthesized in a simulated prebiological environment, have many of the properties of Oparin's coacervates (which are unlikely to be "prebiological", as they can only be synthesized from pre-existing proteins).

The first three chapters deal briefly with the historical, cosmological and geological backgrounds. These aspects of the origins of life are common to most of the new books on the subject, but Fox and Dose have succeeded in bringing out many points of view absent from the rival volumes. Even in the historical section, where the work of A. L. Herrera receives special mention, they have succeeded in developing a distinctive philosophy. The theories of Fox and Dose are, of course, controversial. They champion thermal energy as the power for the final act of what they call "protobiogenesis", and they postulate as a site for this event a volcanic environment. In chapter 3, they consider energy sources, and make an eloquent case for seeking sources which are applied not in the atmosphere, but in lithosphere or hydrosphere. This argument will appeal to the geologist, who is used to finding that most of the energy that powers geological evolution is supplied from inside the Earth, and not from the much greater sources of radiant energy that come from outside. The Earth captures only a small part of this radiant energy, some through the agency of the weather, and the rest through the activities of living organisms which would not have been present before life had formed.

In chapter 7, they return to the theme of energy and environment and here they again enter the controversial lists, and claim that their ideas typify not reductionism, common to most biochemical reasoning, but the "constructionistic" consideration of evolving micro-systems.

The three preceding chapters deal in turn with micromolecules, with macromolecules, and with protocells. Here, on their own ground, the authors really

do communicate to their readers the excitement and enthusiasm which have been such characteristics of their work. Here, brought together for the first time, is a really authoritative and up-to-date account of the Fox hypothesis. It is illustrated with a magnificent set of micrographs, including time-lapse series, which, although many have been published before, are good to have collected together.

Four shorter chapters, on optical activity, on protein sequence, on fossils, and on extraterrestrial problems, conclude the volume. These chapters are useful in that in places the authors can bring cogent criticism to bear on the work of others, but, like the first three chapters, they lack the authority and the immediacy which are such characteristics of the central core of their work. And their enthusiasm is infectious. A special bonus comes in the last three pages. Here, in an appendix, is a recipe for home-made protocells! Readers are encouraged not only to make them for themselves, but also to feed them and watch them grow and divide!

The book is certain to be criticized, for it is much prejudiced in favour of the theories that the authors themselves have so often championed in their experiments. But there are, to my knowledge, at least four other comprehensive books on the origins of life at the moment in press, and we should very soon be able to range against each other all the rival models. Perhaps, in their enthusiasm, Fox and Dose are in danger of creating the mistaken impression that their protocells are almost already "alive". The student who defines life in functional terms may be perplexed, for in the laboratory these protocells absorb food from the environment, grow and multiply by fission. Is this not life? The answer is, of course, very firmly in the negative, for these activities are not spontaneous, they are induced by environmental changes, by (for example) increasing the pH. And in morphological and chemical terms, the differences are even greater. The membranes of protocells are chemically quite distinct from any living cell membrane, and we still await experiments in which protocells have been persuaded to incorporate nucleic acids.

Despite this danger of over-enthusiasm, the protocell hypothesis is such a vital element in the present debate on the origin of life, and it is set out so well, that the volume must surely become compulsory reading for everyone interested in the topic. The book does the publishers credit: it is well produced and reasonably priced. And it is quite the most exciting account that we yet have of what must surely be the most exciting event in the whole history of the Universe.

P. C. SYLVESTER-BRADLEY

Boundary Erosion

Meaning and Control: Essays in Social Aspects of Science and Technology. Edited by D. O. Edge and J. N. Wolfe. Pp. x+274. (Tavistock: London, February 1973.) £4.

ARTHUR KOESTLER, I suppose, might have it that making a book of the papers presented at an international conference is like trying to provide a call girl service by post. Personal contact not being transmissible by the Gutenberg technology, how much point is there in what remains?

If the quality of individual contributions were all that mattered, this volume would pass muster without question. One is entitled, however, to look for more: the coherent development of a theme. The editors suggest that metaphor, and the social context of its use and abuse, form such a theme; but I doubt whether many readers will perceive it as such, once past the first two papers, in which first D. C. Bloor and then Edge deal explicitly with it.

Perhaps the strongest recurrent thread is the attempt to erode boundaries between science and other aspects of human endeavour. Bloor makes the point plainly in his transparently lucid attack on Oxford linguistic philosophers: it is for "boundary maintenance"—for trying to see different spheres of competence as autonomous—that he berates them. Harry Kay points out that, though in general the medium of technology is "more penetrating than any of its hardware", it has not effectively permeated our educational structure; most new machines are not being well integrated into teaching. Eric Trist traces to the dialectic between the "scientific" and the "human relations" approaches to management the newer attempts to organize the technology-people interface; "only the socio-technical whole could be effectively optimized", and principles for job design should explicitly take this into account. Frank Bechhofer's problem regarding shop-floor behaviour is similar, and F. R. Bradbury makes matters specific with regard to the chemical industry.

W. H. G. Armytage wittily traces technocracy through a century and a half. Lewis Gunn analyses the way in which MinTech, "lean and purposeful" in 1965 and concerned with the planned promotion of selected research and development (R and D) programmes, rapidly grew by accretion to become a broadly based sponsoring department for industry. Edwin Mansfield gives a useful summary of his research findings on R and D management, and Wolfe adds a note on cost escalation. R. W. Davies shows how in Soviet Russia, as in the West, there have been attempts to bring R and D closer to users. A brief but cogent paper by

Christopher Freeman deals with R and D for less developed countries.

Although it is hardly possible to deny that issues arising from the science-society relationship form just about the most important group of problems facing mankind, the point still needs making in academic circles, and it is good to see it made in a volume of such high calibre. Some of the papers have suffered from the three-year lag between conference and publication, and some of the newer areas of frontier activity like environment, pollution and conservation are barely mentioned.

F. R. JEVONS

Prime Number Problems

The Distribution of Prime Numbers: Large Sieves and Zero-Density Theorems. By M. N. Huxley. Pp x+128. (Clarendon Press: Oxford; Oxford University Press: London, 1972.) £6.50.

ORIGINALLY, a large sieve was a result expressing in quantitative form the fact that a sufficiently dense integer sequence is well distributed in most arithmetic progressions modulo most (small enough) primes. Nowadays the name covers also a class of allied inequalities for averages of trigonometric and character sums; such inequalities have assumed in recent years increasingly sharp and comprehensive forms, and have led to significant progress in the notoriously difficult study of prime numbers. In 1967 there appeared *Multiplicative Number Theory* (Markham, Chicago) in which the late Professor H. Davenport described the early stages of these developments by ending an exposition of classical prime number theory with a simplified account of Bombieri's now well-known result on the distribution of primes in arithmetic progressions. More recently (1971) H. L. Montgomery's *Lectures on Multiplicative Number Theory* (Springer) have traced the important developments since then (most of these, incidentally, due to himself); and now Huxley's monograph takes the story almost up to the present. Dr Huxley tends to follow Davenport (to whose memory the book is dedicated) in the organization and choice of material; but he is able, of course, to give an account of much more powerful large sieve techniques and of superior results about the zeroes of ζ - and L-functions. He concludes with an account of Vinogradov's famous three primes theorem, based on Montgomery's treatment of the associated trigonometric sums, and with his own result for gaps between consecutive primes, which refines a technique of Montgomery and is currently the "world record", after many years of very slow progress. Huxley is a leading expert in the field, and he shows this in many ways in the course of the book. One of

the ways, unfortunately, is an excessively condensed presentation which, together with some inaccuracies and inadequate references to the ramifications of the various topics introduced, diminishes the value of his book. Individual chapters are heralded by thoughts from Winnie the Pooh, and there are corners of the world where these will be found harder to understand than the chapters themselves. Although workers in the field will be glad to have the latest developments available so soon, they will find reading the book very difficult, and they may well be discouraged from purchasing it at such an outrageously high price.

H. HALBERSTAM

Molecular Spectroscopy

Molecular Spectroscopy: Modern Research. Edited by K. Narahari Rao and C. Weldon Mathews. Pp. xiii+422. (Academic: New York and London, May 1972.) \$25.

THIS volume was issued to commemorate the twenty-fifth anniversary of the Columbus Symposium on Molecular Structure and Spectroscopy, a symposium held annually in the United States at the Ohio State University. The book contains a mixture of review articles, invited talks from the 1970 symposium, and data compilations, though these three categories are not mutually exclusive. Because the material in this volume is so heterogeneous, prospective readers wishing to decide how many articles are closely allied to their own research interests have no recourse but to scan a list of contents. Such a list, with somewhat abbreviated titles, is given in the next paragraph.

One would probably classify as review articles the sections on: microwave spectroscopy (Morino and Saito), rotational levels of free radicals (Carrington), high-resolution IR of planetary atmospheres (Fox), vibration-rotation structure in asymmetric- and symmetric-tops (Mills), electronic spectroscopy of polyatomics (Innes), nuclear hyperfine structure in diatomic electronic spectra (Dunn), IR and UV matrix studies of free radicals and molecular ions (Milligan and Jacox), Fourier spectroscopy (Sanderson), grille spectrometers (Moret-Bailly), and large IR plane gratings (Rao). Written versions of 1970 invited talks include the sections on: Lamb-dip hyperfine spectrum of I_2 (Bunker), rotational energy transfer from MW line broadening (Boggs), rotational spectra of molecules with two internal degrees of freedom (Dreizler), interstellar molecules (Rank), rotational line strengths: the $O_2^+b^4\Sigma_g^- - a^4\Pi_u$ system (Zare) energy transfer research in I_2 (Steinfeld), and biprotonic phototautomerism, excimer

formation, and proton tunnelling in DNA (Kasha, Horowitz, and El-Bayoumi). Finally, the data compilations include: molecular IR laser emissions (Rao and Mantz), and twelve tables of physical constants, conversion factors, wavelength standards, and so on.

There is no question that each of these contributions represents, in its own way, an interesting, enlightening and useful treatment of selected subject matter by a recognized authority in the field. In my own laboratory, those who read the volume all found one or more particularly valuable articles. On the other hand, readers also found article(s) they felt might well have been omitted. Unfortunately, there was no general agreement in selecting individual articles for praise or criticism.

The most attractive general feature of the book to me is the set of excellent longer review articles with large numbers of reference citations, which will be useful for the next several years to anyone beginning work on a problem in one of the areas covered. The line lists and tabulations of constants are also convenient, though they are often accessible elsewhere in the literature.

Perhaps the most severe criticism of the book which comes to mind concerns not the individual articles themselves, but rather the collection as a whole. I am not in favour of the proliferation of random review volumes and/or conference proceedings such as this, which offend one's sense of order and complicate one's literature searches by not fitting neatly into the format of already existing periodic scientific communication channels.

JON T. HOUGEN

Oral Biology

The Marmoset Periodontium in Health and Disease. By B. M. Levy, D. Dreisen and S. Bernick. Pp. 89. (S. Karger: Basel, London and New York, 1972.) 39 Sw. francs; £4.30; \$10.

THIS, the first volume in a series entitled *Monographs in Oral Science*, contains no editorial statement about the scope and aim of the series; nevertheless, the need for animal models to study prevalent dental disease such as gingivitis and pyorrhoea is sufficient reason for this topic to be chosen to initiate the series.

The marmoset, or "manikin", is susceptible to periodontal disease, especially in captivity. Its small size, near-human dentition, and ready availability have made it a most suitable subject for the intensive study of periodontal biology and the reaction of dental supporting tissues to environmental change and injury.

The monograph, in two principal parts, begins with a detailed descrip-

tion of the development of deciduous and permanent dentitions of the marmoset, the structure of the adult periodontium, and its change with increasing age. The text is crisp, and the black-and-white illustrations comprehensive and clear. A monograph on comparative anatomy gains immeasurably by frequent, apposite references to the human situation. That this booklet generally fails to draw these comparisons diminishes its value as a reference work. The deficiency is remedied in the second part devoted to the initiation and extension of periodontal disease. In discussing the role of the external and internal oral environment in the ecology of the periodontium, convincing parallels are drawn between the human and the non-human primate model.

The role of bacteria, viruses, dental plaque and saliva, all key factors in the external environment, has been examined and compared with their suspected role in periodontal disease in man. Dietary changes, vitamin deficiency, and endocrine imbalance have all been employed as tools to influence the internal environment of the periodontium. Results from these valuable studies in the marmoset support Frenkel's contention that "Spontaneous models are most useful to provide inductive hypotheses concerning disease processes in man". If the contents of this slim volume are an indication of things to come, then the series will be of the utmost significance to oral biologists.

D. E. POSWILLO

Human Neurophysiology

Neurophysiology Studied in Man. Edited by G. G. Somjen. (Proceedings of a Symposium held in Paris at the Faculté des Sciences, July 1972.) Pp. xi+487. (Excerpta Medica: Amsterdam, 1972.) Dfl. 108; \$33.75.

THIS symposium, with about ninety contributors, contains as its most distinctive part a great many observations relevant to normal neurophysiology that were made as incidents in treating patients, and could not have been made without the opportunities provided by therapeutic or diagnostic procedures. Papers within this field make up nearly half of the book, and cover the field so widely that the book is quite a good guide to its literature.

Another large part of the book (also nearly half) describes experiments on normal human volunteers. This field is too large for there to be any possibility of covering it in half a symposium, and papers within it look almost randomly selected. They include, I think, the best and the worst in the whole symposium.

Two or three papers are primarily about diseases, and hardly touch neurophysiology.

G. S. BRINDLEY

CORRESPONDENCE

HeLa

SIR,—I would like to thank all those who responded to my letter (*Nature*, **242**, 144; 1973) especially Dr Howard W. Jones, jun., of the Johns Hopkins Hospital. He drew my attention to a publication¹ which leaves no doubt that HeLa cells were named after Henrietta Lacks. The mother of five children, she died less than eight months after her tumour was diagnosed at the age of thirty-one. Others have sought to correct my grammar; to one of these I would point out that the feminine noun "negress" was deliberately avoided as it is known to be offensive to some, and to another that it is just an accepted anomaly of our language that a person's name (singular) is invariably two or more names (plural). I was amused by the suggestion that Gey named HeLa after a favourite film star but it does not fit the facts — Hedy Lamarr is a caucasienne so she, along with Helen Lane, Helga Larsen, Heather Langtree and other unlucky guesses, must now withdraw as gracefully as they can.

Yours faithfully,

J. DOUGLAS

*Department of Applied Biology,
Brunel University,
Kingston Lane, Uxbridge*

¹ Jones, H. W., McKusick, V. A., Harper, P. S., and Kuang-Dong Wu, *Obster. Gynecol.*, **38**, 945 (1971).

Scientists' Careers

SIR,—It is a pleasure to notice as a common strand running through your

excellent group of articles on "Careers for Scientists" (*Nature*, **242**, 375; 1973) the recognition of science courses as an education fitting the graduate for an almost unlimited range of work rather than as a narrow vocational training. Naturally somebody of my persuasion would have liked to see the point emphasized even more, would have liked to see it everywhere accepted with joy rather than as, here and there, with a somewhat regretful attitude, would have liked all the articles to echo Dainton's admonition to the universities to adapt their teaching accordingly. But, above all, employers must more generally look at scientists not as a unique species fitted only for laboratory jobs, but as educated people who must be considered with all others for every task requiring intellectual ability.

It is, however, not only at the start of his career that the scientist should look beyond the confines of the laboratory bench job. An increased mobility of experienced research scientists, just as of raw graduates, would benefit them, their employers, society at large, and the graduates who follow them. My Task Force is trying to assist this mobility through arranging interchanges so that the scientist can experience a new kind of work without having to give up his earlier career irrevocably.

Yours faithfully,

HERMANN BONDI

*Task Force on Interchange of Scientists,
Civil Service Department,
Whitehall, SW1*

Looting Art Treasures

SIR, — As an Italian-born American citizen I feel that I belong to the looting and the looted party in the case of the Greek vase bought by the Metropolitan Museum in New York from a dealer resident in Rome (*Nature*, **242**, 155; 1973). From this vantage point I am not so sure that the singling out of the Metropolitan Museum is wholly justified. It seems to me that all nations at one time or another have practised looting of art objects on a grand scale. From the Bronze Horse of the Pala D'oro in St Mark to the tapestries of the Duke of Burgundy in Berne, and from Lord Elgin's marbles to the illuminated manuscript in the Bibliothèque Nationale Française, it seems to me the looting has been the rule rather than the exception for several centuries. (I must say that if I have omitted other nations it is not because they are innocent, but simply that their looting has been inferior in quality rather than quantity.)

The only trouble with the Metropolitan Museum is that they arrive with a wolf's hunger to a banquet that is already over; or, to put it in biblical terms "whosoever is without sin, let him cast the first stones" (or should I say "the first marbles"?).

Yours faithfully,

RENATO BASERGA

*Department of Pathology,
Temple University Health Sciences
Center,
Philadelphia, Pennsylvania 19140*

Obituary

Academician A. N. Tupolev

ACADEMICIAN ANDREI NIKOLAEVICH TUPOLEV, the famous Russian aircraft designer, died on December 23, 1972, after a long illness.

Tupolev, who was born on November 10, 1888, the son of a notary, was educated at the *gymnasium* in Tver' and then at the Moscow Higher Technical Institute, where he studied under N. E. Zhukovskii, the aviation pioneer.

In 1918, after graduating, Tupolev assisted Zhukovskii in the organization of the Central Institute of Aerohydro-

dynamics, becoming one of its Deputy Directors, and, in 1922, Director of its Design Bureau. From then on, Tupolev became increasingly the most prominent figure in the Soviet aviation industry, designing in all some 120 types of aircraft.

During the 1920s he carried out extensive research into the use of duraluminum in aircraft construction, and is widely regarded as one of the pioneers of all-metal aircraft. In 1934 he constructed an experimental eight-engine passenger aircraft, the "Maxim Gorky" with a wing span of some 65 m and weighing 40 tons. This aircraft made a

number of successful test flights but crashed in 1935, an accompanying fighter plane being, apparently, responsible for the disaster.

In 1936, Tupolev visited Germany and the USA to study foreign aircraft construction. Shortly after his return to Russia, during the Stalinist purges, he was accused of "divulging aviation secrets" and sentenced to forced labour. During his imprisonment he designed a twin-engined divebomber, the Tu-2 which was put into production in 1939 and formed an important part of the Soviet air arm during World War II. Shortly after designing this aircraft,

Tupolev was released and returned to work at the Design Bureau of the Central Institute of Aerohydrodynamics, where he remained for the rest of his working life, gradually acquiring a great number of titles and awards, including Member of the Academy of Sciences of the USSR (1953), Designer General to the USSR Ministry of the Aviation Industry (1965), three Stalin Prizes (now State Prizes), one Lenin Prize, the Order of Lenin (8 times), the Order of the Red Banner of Labour (twice), the order of the Red Star (twice) and the title of Hero of Socialist Labour (twice), this last being the highest Soviet civilian award.

Among the most notable aircraft designed by Tupolev during the course of his long career were the single-engined ANT-25 which in 1937 made the first non-stop trans-polar flight from the Soviet Union to the USA, the four-engined ANT-6 which landed the personnel of the Soviet North Pole expedition in 1937, the first civil passenger turbo-jet, the Tu-104, and the supersonic passenger aircraft, the Tu-144, which is expected to go into service in 1975.

Andrei Nikolaevich Tupolev, who gave his name to the Tu-series of aircraft, is succeeded as head of the design team by his son, Aleksei Andreevich, who, it is understood, will be in charge of final tests and modifications to the Tu-144.

Announcements

University News

Dr Peter Watt, St Mary's Hospital, Medical School, has been appointed to the Chair of Microbiology at the University of Southampton as from October 1, 1973.

Miscellaneous

Professor Sir Ernst Chain, Department of Biochemistry, Imperial College, has been awarded the Grand Decoration of Honour in Gold for his services to the Republic of Austria.

Dr J. H. Baxendale, Department of Chemistry, University of Manchester, has been awarded the Weiss Medal for his contributions to radiation research.

The following have been awarded the **Linnean Society Gold Medal**: **Professor G. L. Stebbins**, University of California, and **Professor J. Z. Young**, University College London.

Dr Ursula K. Duncan has been awarded the H. H. Bloomer award by the Linnean Society of London.

Mr N. L. Falcon has been awarded the Founder's Medal of the Royal Geographical Society.

Professor E. H. Thompson, University College London, has been awarded the Patron's Medal of the Royal Geographical Society.

Dr A. H. Chilver, Cranfield Institute of Technology, has been appointed chairman of the committee of Education and Training at the Department of Trade and Industry.

Mr H. Surtees, Easams Limited, has been appointed chairman of the committee of Materials Handling at the Department of Trade and Industry.

Mr N. Sheperd, Dept of Trade and Industry, has been appointed chairman of the committee of Information and Publicity at the Department of Trade and Industry.

Dr Sidney Passman, US National Science Foundation, has been appointed director of the division of Scientific Research and Higher Education at Unesco in Paris.

Errata

IN the article "Comments on the Isolation, Identification and Synthesis of a Specific-Behaviour-Inducing Brain Peptide" by Avram Goldstein (*Nature*, **242**, 60; 1973) the first sentence of paragraph 2 should read: "The long controversy over the claims for transfer of learned behaviours was reviewed by W. W. Stewart² that involved a rebuttal by Ungar *et al.*".

IN the article "Isolation of the Islets of Langerhans for Transplantation" by D. R. Thomas, M. Fox and A. A. Grieve (*Nature*, **242**, 258; 1973) paragraph 1, line 5 and following, should read: "One of the major problems has been to overcome pancreatic exocrine digestion. Pancreatic duct ligation ("Banting pancreas") before transplantation has been performed with this in view."

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

Bulletin of the British Museum (Natural History). Entomology. Vol. 27, No. 4: Revisional Notes on African *Charaxes* (Lepidoptera: Nymphalidae), Part VIII. By V. G. L. van Someren. Pp. 215-264+12 plates. (London: British Museum (Natural History), 1972.) £5. [12]

Agricultural Research Council. Meat Research Institute Annual Report 1971-72. Pp. 105. (London: Agricultural Research Council, 1973. Obtainable from HMSO.) £1.40 net. [12]

New Developments in Manufacture and Use of Liquid Fertilisers. By A. V. Slack and F. P. Achorn. Pp. 51. (London: The Fertiliser Society, 1973.) [12]

Commonwealth Forest Institute. Paper No. 45: The Planning and Evaluation of Forestry Projects. By G. R. Watt. Edited by J. J. MacGregor. Pp. xii+83. (Oxford: Commonwealth Forestry Institute, University of Oxford, 1973.) £2 (postage 8p). [12]

The Medical Research Council of Ireland. Annual Report for the year ended December 31, 1971. Pp. 100. (Dublin: The Medical Research Council of Ireland, 9 Clyde Road, 1973.) 25p. [22]

Journal of Environmental Management, Vol. 1, No. 1, January 1973. Pp. 1-84. Published quarterly. Vol. 1, 1973: UK £6.60, overseas £7.60. (London and New York: Academic Press, Inc., 1973.) [22]

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